JAB 339

#17

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

Inventors : Josephus Brugmans, William Pollack, Paul A. J. Janssen, and Daniel Tripodi

U.S. Patent No.: 4,584,305

Issued: April 22, 1986

For: AIDING THE REGRESSION OF NEOPLASTIC DISEASE WITH 2,3,5,6-TETRAHYDRO-6-PHENYLAMIDAZO[2,1-b]THIAZOLE

Hon. Commissioner of Patents and Trademarks
Box Patent EXT
Washington, D.C. 20231

TRANSMITTAL LETTER

Dear Sir:

Transmitted herewith is an Application for Extension of Patent Term under 35 U.S.C. 156 in the above-identified patent.

Please charge the $550.00 application fee to Deposit Account No. 10-750 in the name of Johnson & Johnson.

The Commissioner is hereby authorized to charge any additional fee which may be required or to credit any overpayments to Deposit Account 10-750.

Two copies of this letter are enclosed.

Respectfully submitted,

Charles J. Metz

Charles J. Metz
Registration No. 20,359
Attorney for Applicants

Johnson & Johnson
One Johnson & Johnson Plaza
New Brunswick, New Jersey 08933-7003
(201) 524-2814

July 31, 1990

JAB 339

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

Inventors : Josephus Brugmans, William Pollack, Paul A. J. Janssen, and Daniel Tripodi

U.S. Patent No.: 4,584,305

Issued: April 22, 1986

For: AIDING THE REGRESSION OF NEOPLASTIC DISEASE WITH 2,3,5,6-TETRAHYDRO-6-PHENYLAMIDAZO[2,1-b]THIAZOLE

Certificate

"Express Mail" mailing number B 81831552

Date of Deposit August 1, 1990

I hereby certify that this complete Application for Extension of Patent Term including the Transmittal Letter and all Exhibits and Attachments named therein is being deposited in duplicate with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patent and Trademarks, BOX PATENT EXT, Washington, D.C. 20231.

Charles J. Metz
(Typed or printed name of person mailing paper or fee)

Charles J. Metz
(Signature of person mailing paper or fee)

JAB 339

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

Inventors : Josephus Brugmans, William Pollack, Paul A. J. Janssen, and Daniel Tripodi

U.S. Patent No.: 4,584,305

Issued: April 22, 1986

For: AIDING THE REGRESSION OF NEOPLASTIC DISEASE WITH 2,3,5,6-TETRAHYDRO-6-PHENYLAMIDAZO[2,1-b]THIAZOLE

Hon. Commissioner of Patents and Trademarks
Box Patent EXT
Washington, D.C. 20231

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. 156

Dear Sir:

Applicant Janssen Pharmaceutica N.V., a Belgian business corporation, represents that it is the assignee of the entire interest in and to Letters Patent of the United States No. 4,584,305 granted to Josephus Brugmans, William Pollack, Paul A. J. Janssen, and Daniel Tripodi on April 22, 1986 by virtue of an assignment to Janssen Pharmaceutica N.V. recorded in the United States Patent and Trademark Office on February 5, 1986, at reel 4513, frame 0277.

Applicant hereby submits this Application for Extension of Patent Term under 35 U.S.C. 156 and provides the following information according to the relevant regulations set out at 37 C.F.R. 1.710 et seq. The numbering of the following paragraphs corresponds to the numbering of the requirements for an application set forth in 37 C.F.R. 1.740.

The approved product is levamisole hydrochloride. Levamisole hydrochloride is approved as safe and effective for use as adjuvant treatment in combination with fluorouracil after surgical resection in patients with Dukes' stage C colon cancer. The complete identification of levamisole is the following:

Chemical Name:

S-(-)-2,3,5,6-tetrahydro-6-phenyl-imidazo[2,1-b]thiazole hydrochloride

also known as:

the levo enantiomorph of 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole hydrochloride

Generic Name:

levamisole hydrochloride

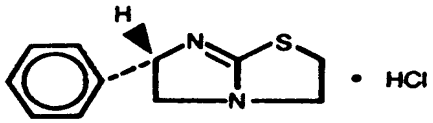
Manufacturer's Research Number:

R 12,564

Manufacturer's Registered Trade Name:

Ergamisol

Physical Structure:



Characteristics:

- melting point 227-229°C
- molecular formula $C_{11}H_{12}N_2S \cdot HCl$

(6)

The complete identification of the patent for which this extension is being sought is as follows:

Inventors: Josephus Brugmans, William Pollack, Paul A. J. Janssen, and Daniel Tripodi

Patent Number: 4,584,305

Date of Issue: April 22, 1986

Date of Expiration: April 22, 2003

(7)

A copy of the patent for which an extension is being sought is appended hereto as Exhibit A.

(8)

The records of the undersigned do not indicate that any disclaimers or re-examination certificates were issued in the patent identified in paragraph (6). A letter requesting a Certificate of Correction was filed in the Patent and Trademark Office by Attorney Goeffrey G. Dellenbaugh on June 11, 1986, and a modified Certificate of Correction was issued on September 30, 1986, copies of both the letter and the modified Certificate of Correction are appended hereto as Exhibits B and C, respectively. On September 20, 1989, a receipt for Maintenance Fee Payment was mailed from the Patent and Trademark Office, a copy of which is appended hereto as Exhibit D.

(2)

The approved product was subject to regulatory review under the Federal Food, Drug and Cosmetic Act, Section 505 (21 U.S.C. 355)

(3)

The approved product received permission for commercial marketing or use under Section 505 of the Federal Food Drug and Cosmetic Act (21 U.S.C. 355) on the following date:

June 18, 1990.

(4)

The approved product is a human drug product containing levamisole hydrochloride as the active ingredient. See paragraph (1) above for a more complete description of levamisole hydrochloride. Levamisole hydrochloride has not been previously approved for commercial marketing or use as a human drug product under Section 505 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 355). Levamisole hydrochloride has previously been approved under Section 512 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b) for commercial marketing or use as a veterinary product.

(5)

This application for extension of patent term under 35 U.S.C. 156 is being submitted within the sixty (60) day period permitted for submission pursuant to 37 C.F.R. 1.720(f). The last day on which this application could be submitted is August 17, 1990.

(9)

United States Patent Number 4,584,305 claims a method of aiding the regression and palliation of neoplastic diseases (cancer) which comprises the systemic administration to human and animal subjects of an effective anti-neoplastic amount of a member selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole and the therapeutically active acid addition salts thereof in a pharmaceutical carrier. Claims 1, 2, 10, and 11 of U.S. Patent 4,584,305 each read on a method of using the approved product for the treatment of Dukes' C colon cancer. The following is a demonstration of the manner in which each of such patent claims reads on a method of using the approved product:

The approved product is the hydrochloride acid addition salt of the levo enantiomorph of 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole. In each of the four claims, Nos. 1, 2, 10, and 11, select the levo enantiomorph of 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole and the hydrochloride acid addition salt of such levo enantiomorph. That the levo enantiomorph of 2,3,5,6-tetrahydro-6-phenyl-imidazo[2,1-b]thiazole is clearly within the scope of the patent's claims is clear from the disclosure of the patent, for example, see Col. 1, lines 22-28, and Col. 2, lines 55-56.

(10)

The relevant dates and information pursuant to 35 U.S.C. 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review periods are as follows:

(a) The Investigational New Drug (IND) application [i.e., the application for exemption under subsection (i) of section 505 of the Federal Food, Drug, and Cosmetic act (21 U.S.C. 355(i)] under which the clinical studies were performed which were referenced in the New Drug Application (NDA) referred to in sub-paragraph (10)(b) below was filed on February 24, 1977, by the National Cancer Institute of the National Institutes of Health, a federal agency within the Department of Health & Human Services (hereinafter "NCI"), the application was effective as of April 8, 1977, and it was assigned IND # 13,266.

(b) The New Drug Application (NDA) [i.e., the application for approval under subsection (b) of section 505 of the Federal Food, Drug, and Cosmetic act (21 U.S.C. 355(b)] for the use of levamisole hydrochloride as adjuvant treatment in combination with fluorouracil after surgical resection in patients with Dukes' stage C colon cancer was submitted on October 31, 1989, by Janssen Research Foundation, and was assigned NDA # 20-035.

(c) The date on which NDA # 20-035 was approved was June 18, 1990.

(11)

(a) A brief description of the significant activities and dates applicable to such activities undertaken by or on behalf of the marketing applicant during the applicable regulatory review with respect to the approved product is set forth below in subparagraphs (11)(b) and (11)(c). In this regard, it should be noted that the Applicant herein is Janssen Pharmaceutica N.V., a corporation of Belgium and a wholly-owned subsidiary of Johnson & Johnson, a corporation of New Jersey, U.S.A. While U.S. Patent 4,584,305 has been and is now owned by Janssen Pharmaceutica N.V., the IND and NDA submissions and activities described herein were undertaken by NCI and by the Janssen Research Foundation, a Delaware corporation, which is a wholly owned subsidiary of Johnson & Johnson. (The Janssen Research Foundation will occasionally be referred to hereinafter as "JRF".) All IND and NDA activities undertaken as described below in subparagraphs (11)(b) and (11)(c) were carried out with the full and complete permission of Janssen Pharmaceutica N.V. and Johnson & Johnson.

(b) Relevant Activities Under IND 13,266

February 24, 1977 - IND submitted to FDA by NCI.

April 8, 1977 - Effective date of IND 13,266.

July 18, 1978 - Protocol for North Central Cancer Treatment Group ("NCCTG") submitted to FDA by NCI.

The NCCTG Protocol was a study of 401 patients with resected stages B and C colorectal cancer, and was designed to evaluate the effectiveness of levamisole hydrochloride and the

combination of levamisole hydrochloride and fluorouracil in the treatment of such patients. The study is reported by Laurie et al., in SURGICAL ADJUVANT THERAPY OF LARGE-BOWEL CARCINOMA: AN EVALUATION OF LEVAMISOLE AND THE COMBINATION OF LEVAMISOLE AND FLUOROURACIL, Journal of Clinical Oncology, Vol 7, No. 10 (October), 1989: pages 1447-1456, a copy of which is appended hereto as Exhibit E. The study concluded that:

> "Levamisole plus 5-FU [fluorouracil] ... reduced cancer recurrence in comparison with no adjuvant therapy." (Page 1447.)

However, the authors also recommended further confirmation (page 1455).

November 7, 1984 - Intergroup 0035 Protocol submitted to the FDA by NCI.

The Intergroup 0035 study was a study of 1296 patients with resected colon cancer. The study was designed to confirm the findings of the NCCTG study. This study is reported by Moertel et al. in LEVAMISOLE AND FLUOROURACIL FOR ADJUVANT THERAPY OF RESECTED COLON CARCINOMA, The New England Journal Of Medicine, Vol. 322, No. 6, February 4, 1990, pages 352-358. A copy of this article is appended hereto as Exhibit F. The authors conclude that:

> "We conclude that adjuvant therapy with levamisole and fluorouracil should be standard treatment for Stage C colon carcinoma." (Page 352.)

Early September 1989 - Steering committee for the Intergroup 0035 study met and discussed the results of the study. It was concluded that the results were meaningful and that the conclusion of the NCCTG study had been confirmed.

September 29, 1989 - Pre-NDA meeting at FDA with Dr. Moertel (the lead author of the New England Journal of Medicine article cited above) and representatives from the Janssen Research Foundation and NCI in attendance. Dr. Moertel described the results of the Intergroup 0035 study.

Mid-October, 1989 - Data tapes from the Intergroup 0035 study were made available to JRF from NCI. The data from these tapes were used by JRF in preparing the clinical report that was included in the submission of NDA 20-035.

(c) Relevant Activities Under NDA 20-035

1.	Date Submitted to FDA by Janssen Research Foundation	October 31, 1989
2.	Date Received	November 1, 1989
3.	Advisory Committee Meeting	February 1, 1990
4.	Chemistry Comments	February 13, 1990
	Questions on reference standard, synthesis of drug substance, specifications for drug substance and drug product, packaging material, and stability.	
	Responses	March 14, 1990
5.	Environmental Assessment Comments	February 16, 1990
	Request for test data on environmental fate, health effects, and environmental effects.	
	Response	April 2, 1990
6.	Medical Comments on PI (Package Insert)	March 12, 1990
	Additions to sections on precautions, pregnancy, adverse reactions, and treatment; add section on pediatric use.	
	Response	April 11, 1990
7.	Pharmacokinetics Comments	March 26, 1990
	Request commitment for additional bioavailability, pharmacokinetic, and drug interaction studies.	
	Response	April 11, 1990

8.	Chemistry Comments	March 29, 1990
	Questions on synthesis and specifications for tetramisole hydrochloride*, moisture limits, and packaging.	
	Partial Response	April 3, 1990
	Response	April 11, 1990
9.	Chemistry Comments	April 20, 1990
	Questions on impurities and sources of tetramisole hydrochloride, stability, packaging, and labeling.	
	Response	May 10, 1990
10.	Medical Comments on PI	May 11, 1990
	Change in section on carcinogenicity, mutagenicity, and impairment of fertility.	
	Response	May 14, 1990
11.	Safety Update	May 21, 1990
	Report from Chinese medical journal describing cases of "encephalopathy".	
12.	Medical Comments on PI	May 31, 1990
	Addition of encephalopathy-like syndrome to adverse reactions.	
	Response	June 1, 1990
13.	Medical Comments on PI	June 15, 1990
	Changes to the following sections: mechanism of action, warnings, precautions, adverse reactions, and overdosage.	
	Response	June 18, 1990
14.	Chemistry Comments	June 18, 1990
	Commitment made to attempt to obtain synthesis route of tetramisole hydrochloride from suppliers.	
	Response	June 18, 1990
15	NDA Approved	June 18, 1990

---

*Tetramisole is the racemic mixture of the levo and dextro enantiomorphs of 2,3,5,6-tetrahydro-6-phenylimidazo-[2,1-b]thiazole hydrochloride. As was pointed out in Paragraph (1) above, levamisole is the levo enantiomorph of 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole hydrochloride.

(12)

Applicant is of the opinion that U.S. Patent 4,584,305 is eligible for extension under 35 U.S.C. 156.

The length of extension claimed in the present application is one year, one month, and 27 days from April 22, 2003 to June 18, 2004. The requested one year, one month, and 27 day extension is the maximum permitted by the limitations of 35 U.S.C. 156(g)(4)(C). The requested extension does not exceed the fourteen year maximum from the date of approval, i.e. to June 18, 2004, imposed by the limitation of 35 U.S.C. 156(c)(3). This extension is supported by the regulatory review period for the approved product, which exceeds the requested extension.

(13)

The Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought in the present application for extension.

(14)

Accompanying this application is a transmittal letter which requests that the required fee for the present application for extension to be charged to Deposit Account Number 10-750.

(15)

The name, address, and telephone number of the person to whom inquiries and correspondence relating to the present application for patent term extension are to be directed is as follows:

Address: Robert L. Minier
Johnson & Johnson
One Johnson & Johnson Plaza
New Brunswick, New Jersey 08933-7003

Telephone Calls: Charles J. Metz
(201) 524-2814

(16)

A duplicate of this application is enclosed. The required certification is appended hereto as Exhibit G.

(17)

The Declaration required by 37 C.F.R. 1.740(b) is attached hereto as Exhibit H.

Respectfully submitted,

Charles J. Metz

Charles J. Metz
Registration No. 20,359
Attorney for Applicant

Johnson & Johnson
One Johnson & Johnson Plaza
New Brunswick, New Jersey 08933-7003
(201) 524-2814

July 31, 1990

EXHIBIT A

United States Patent [19]

Brugmans et al.

[11] **Patent Number: 4,584,305**

[45] **Date of Patent: Apr. 22, 1986**

[54] **AIDING THE REGRESSION OF NEOPLASTIC DISEASE WITH 2,3,5,6-TETRAHYDRO-6-PHENYLIMIDAZO[2,1-b]THIAZOLE**

[75] Inventors: **Josephus Brugmans**, Schilde-Antwerp, Belgium; **William Pollack**, Belle Mead, N.J.; **Paul A. J. Janssen**, Vosselaar, Belgium; **Daniel Tripodi**, Westford, Mass.

[73] Assignee: **Janssen Pharmaceutica N.V.**, Beerse, Belgium

[21] Appl. No.: **230,578**

[22] Filed: **Feb. 2, 1981**

Related U.S. Application Data

[63] Continuation of Ser. No. 67,505, Aug. 17, 1979, abandoned, which is a continuation of Ser. No. 944,520, Sep. 30, 1978, abandoned, which is a continuation of Ser. No. 799,893, May 23, 1977, abandoned, which is a continuation of Ser. No. 591,795, Jun. 30, 1975, abandoned, which is a continuation-in-part of Ser. No. 424,030, Dec. 12, 1973, abandoned, which is a continuation-in-part of Ser. No. 281,367, Aug. 17, 1972, abandoned.

[51] **Int. Cl.**[4] **A61K 31/425**

[52] **U.S. Cl.** **514/368**

[58] **Field of Search** 424/270; 514/368

[56] **References Cited**

PUBLICATIONS

Renoux et al., Nature, vol. 240, pp. 217-218 12-13-72.

Primary Examiner—Jerome D. Goldberg
Attorney, Agent, or Firm—Geoffrey G. Dellenbaugh

[57] **ABSTRACT**

Process of aiding regression and palliation of neoplastic disease in animal and human hosts comprising systemic administration to phenylimidazo[2,1-b]thiazole or a nontoxic acid addition salt thereof. Preferably the active ingredient is administered in a sufficient amount to provide dosages over the range from about 1 mg to about 5 mg/kg of body weight of the host.

17 Claims, No Drawings

AIDING THE REGRESSION OF NEOPLASTIC DISEASE WITH 2,3,5,6-TETRAHYDRO-6-PHENYLIMIDAZO[2,1-b]THIAZOLE

This is a continuation of application Ser. No. 067,505, filed Aug. 17, 1979, now abandoned, which in turn is a continuation of Application Ser. No. 944,520, filed Sept. 30, 1978, now abandoned, which in turn is a continuation of Application Ser. No. 799,893, filed May 23, 1977, now abandoned, which in turn is a continuation of Application Ser. No. 591,795, filed June 30,1975, now abandoned, which in turn is a continuation-in-part of Application Ser. No. 424,030, filed Dec. 12, 1973, now abandoned, which in turn is a continuation-in-part of Application Ser. No. 281,367, filed Aug. 17, 1972, now abandoned.

DESCRIPTION OF THE INVENTION

This invention relates to a process of ameliorating neoplastic disease in animal and human hosts. The essential active ingredient utilized in the process in an effective amount is 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole (PIT). This compound is a well known anthelmintic, generically known as tetramisole in its racemic form and as levamisole in the form of the levo enantiomorph. From about 1 mg to about 5 mg/kg body weight of the host, calculated as the base form, is the preferred range for the essential ingredient.

PIT, in base form, may be readily converted to the corresponding therapeutically acceptable acid addition salt form by reaction with an appropriate inorganic acid, such as, for example, hydrochloric, hydrobromic, hydriodic, sulfuric, phosphoric and the like acids, or with an appropriate organic acid, such as, for example, acetic, propionic, glycolic, lactic, oxalic, malonic, tartaric, citric, sulfamic, ascorbic and the like acids. In turn, the salts of formula (I) may be converted to the corresponding base form by conventional treatment with suitable alkali. Of the acid addition salts, the hydrochloride is preferred.

Neoplastic disease, as used herein, is meant to include all types of cancerour growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. For example, the subject compounds may be used in accordance with this invention against such neoplastic disorders as "Lewis Lung 3LL" tumor and pulmonary metastases, methylcholanthrene indeed sarcoma, Maloney leukemia, sarcoma 180 and the like in laboratory animals, for example, mice, and against such neoplastic disorders as shown in New-England J. Med., Vol. 289, P. 354 (1973).

The process of this invention comprises systemically administering to subjects hosting neoplastic disease an effective ameliorating amount of PIT or a therapeutically active acid addition salt thereof preferably admixed with a pharmaceutically acceptable carrier. Such carrier may take a wide variety of forms depending on the form of preparation desired for administration, i.e., oral or parenteral. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, and the like in the case of oral liquid preparations such as suspensions, elixirs and solutions or solid carriers such as starches, sugars, kaolin, lubricants, binders, disintegrating agents and the like in the case of powders, capsules and tablets. Because of their case in administration, tablets and capsules represents the most advantageous oral dosage form, in which case solid pharmaceutical carriers are obviously employed. For parenteral injection, the carrier will usually comprise sterile water, at least in large part, though other ingredients, for example, to aid solubility, may be included. Injectable solutions, for example may be prepared in which the carrier comprises saline solution, glucose, solution or a ixture of saline and glucose solution. Injectable suspensions may also be prepared in which case appropriate liquid carriers, suspending agents and the like may be employed.

It is especially advantageous to formulate the aforementioned pharmaceutical compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form, as used herein, refers to physically discrete units suitable as unitary dosages, each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. Examples of such dosage unit forms are tablets, capsules, pills, powder packets, wafers, teaspoonfuls, tablespoonfuls and the like, and segregated multiples thereof. The amount of active ingredient per dosage unit may range from about 5 mg to about 500 mg, and, preferably, from about 50 mg to about 250 mg.

The dosage of the principal active ingredient (PIT) for the treatment of the particular neoplastic disease may depend on the species and size of the subject being treated; the particular condition and its severity; the particular form of the active ingredient (e.g., soluble salt or less soluble base) and the route of administration. In any case the dose to be used is one nontoxic to the recipient. In general, a dose of from about 1.0 mg/kg body weight up to the nontoxic anthelmintic dose for the particular host can be generally utilized for the treatment of neoplastic disease well. For example, the recommended nontoxic anthelmintic oral dose for tetramisole is about 5 mg/kg in man, about 15 mg/kg in sheep, about 10 mg/kg in cattle, and about 40 mg/kg in chickens; and for levamisole about 2.5 mg/kg in man, about 8 mg/kg in sheep, and about 8 mg/kg in cattle. Expressed as amounts suited for single unit doses, from about 5 to about 500 mg is operable and expedient for most subjects.

In humans, a dose of from about 1.0 mg/kg to about 5 mg/kg, or a daily total dose or from about 50 to about 500 mg given singly or in divided doses embraces the effective range for the treatment of most neoplastic diseases.

Regression and palliation of neoplastic disease are aided by the internal administration of PIT, preferably as the hydrochloride salt of the levo enantiomorph, and pharmaceutical compositions containing same.

As a dosage regimen, the amount of principal active ingredient administered is a sufficient amount to aid regression and palliation of the neoplastic disease in the absence of excessive deleterious side effects of a cytotoxic nature to the host harboring the disease. Specific modes of administration are 150 mg/kg of levamisole or 250–300 mg/kg of tetramisole daily to humans for about 3 to 5 days repeated every 2–3 weeks. In certain instance, continuous daily administration of 100–200 mg/kg of levamisole or 200–300 mg/kg of tetramisole may be maintained over a long period of time, for example 3–6 months.

EXAMPLE I

Injectable solution: A sterile aqueous solution suitable for intramuscular or intravenous use, and containing 250 mg or tetramisole hydrochloride as the active ingredient (A.I.) in each ml, is prepared from the following formulation:

Tetramisole HCl	250 gms
Water for Injection, U.S.P., q.s. ad	1,000 ml

EXAMPLE II

Capsules: 10,000 Hard gelatin capsules, each containing as the active ingredient 150 mg of levamisole hydrochloride, are prepared from the following formulation:

	Grams
Levamisole HCl	1.500
Lactose	500
Starch	150
Talc	150
Calcium Stearate	10

A uniform mixture of the active and supplementary ingredients is prepared and filled into two-piece hard gelatin capsules. The capsules provide satisfactory regression and palliation of neoplastic disease in adults with a regimen of 1 capsule given daily for 3 days, said regimen being repeated every three weeks.

EXAMPLE III

Tablets: 5,000 Compressed tablets, each containing as the active ingredient 150 mg of levamisole hydrochloride, are prepared from the following formulation:

	Grams
Levamisole HCl	750
Starch	75
Dibasic calcium phosphate hydrous	325
Calcium stearate	3.5

The finely powdered ingredients are mixed well and granulated with 10% starch paste. The granulation is dried and compressed into tablets. The oral administration of one tablet a day for 3 days, repeated every 3 weeks, provides satisfactory regression and palliation of neoplastic disease in adult human. The tablets may be sugar coated to mask the tast of the active ingredient.

EXAMPLE IV

The following formulation provides 5 liters of an oral suspension comprising 50 mg of di-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole as the active ingredient (A.I.) per teaspoonful (5 mls):

	Grams
A.I.	25.0
Sucrose	300.0
Dioctyl sodium sulfosuccina	0.5
Bentonite	22.5
Methyl paraben	7.5
Propyl paraben	1.5
Antifoam A.F. Emulsion	0.15
Propylene glycol	52.0
FD & C Yellow #5	0.1
Sodium cyclamate	50.0
Sodium saccharin	5.0
Orange flavor	7.5
Filtered purified water, q.s., ad	5 liters

Dissolve the parabens in the propylene glycol and add this solution to a solution of the sodium cyclamate, sodium saccharin and sucrose in half the water. Suspend the bentonite in hot (about 85° C.) water and stir for 60 minutes. Add the bentonite solution to the former solution.

Dissolve the sulfosuccinate in some water and suspend the A.I. in the resulting solution. Add the Antifoam A.F. Emulsion which had been diluted to a lotion consistency with a minimum amount of water and mix well.

Add the letter suspension of A.I. to the former mixture and mix well. Add the FD&C Yellow #5 dissolved in a small amount of water. Add the orange flavor, q.s. to volume with water, and stir to a homogeneous mixture. Pass the mixture through a colloid mill and fill into suitable containers.

EXAMPLE V

Two-month-old female C57B1/Rho mice were inoculated subcutaneously in the flank with 0.2 ml. of a tumor cell suspension that contained 2.5×10^6 live cells (trypan blue exclusion test) per ml. Three weeks later all mice were killed, the primary subcutaneous tumor excised and weighed, and the lungs examined for metastases that appeared as white nodules against the black substance of normal lung tissue following injection of dilute India ink through the trachea before fixation of the whole lung.

In our experiment, all mice were inoculated on the same day with 5×10^5 live tumor cells suspended in buffered saline, pH 7.2 (Table 1). After 24 hr one group of mice taken at random was treated by a single subcutaneous injection of 0.1 ml. of levamisole dissolved in sterile pyrogen-free saline (0.5 mg kg^{-1}. In a second randomized group treatment by levamisole, 0.5 mg kg^{-1} at each injection, was started 7 days after tumour inoculation when palpable tumor nodules had already developed in all untreated mice. Levamisole treatment in this group was continued every 2 days up to the 17th day, for a total of 6 injections. Controls were left untreated (Table 1).

Findings at autopsies on day 21 after tumo inoculation were statistically analyzed. Table 1 shows that a single injection of 0.5 mg kg^{-1} of levamisole sufficed to completely cure 3 out of 12 mice and significantly ($P=0.01$) inhibited both the primary tumor growth and the number of pulmonary metastases in the 9 other mice of that group. Levamisole treatment also cured 4 out of 10 mice when treatment was started after subcutaneous tumor had developed. In the same group of mice, one additional animal did not demonstrate any lung metastases. Primary tumor and pulmonary metastases were significantly ($P=0.01$) reduced in the remaining mice of that group.

TABLE I

Levamisole Activity on the Primary 3 LL Tumour and Pulmonary Metastases

Group No.	Treatment	No. of mice	Primary tumour: Negative intake	Primary tumour: Geometric mean weight (g) in positive mice	Primary tumour: Confidence interval (0.95)		Lung metastases: Negative mice	Lung metastases: Geometric mean No. in positive mice	Lung metastases: Confidence interval (0.95)	
1	None	10	0	3.70	(3.39–4.01)		0	20.2		(18.2–22.4)
2	0.5 mg kg^{-1} levamisole, day 1	12	3	2.27	(1.43–3.11)	NS	3	3.2	NS	(0.7–5.7)
3	0.5 mg kg^{-1} levamisole, days 7, 9, 11, 13, 15, 17	10	4	1.40	(0.93–1.87)		5	3.0		(1.5–4.5)

The foregoing example demonstrates the activity of levamisole, a simple chemical compound devoid of toxicity (mouse LD_{50} = 121 mg/kg), against the growth of primary subcutaneous 3 LL tumor and against the development of pulmonary metastases.

What is claimed is:

1. A process of aiding regression and palliation of neoplastic disease which comprises the systemic administration to human and animal subjects hosting the neoplastic disease of an effective anti-neoplastic amount of a member selected from the group consisting of 2,3,5,6-tetrahydo-6-phenylimidazo[2,1-b]thiazole and the therapeutically active acid addition salts thereof in a pharmaceutical carrier.

2. A process of aiding regression and palliation of neoplastic disease which comprises the systemic administration to a human hosting the neoplastic disease of from about 1 to about 5 mg/kg body weight of the host of a member selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole and the therapeutically active acid addition salts thereof.

3. A process of aiding regression and palliation of pulmonary metastatic tumor which comprises the systemic adminstration to a host of said tumor of an effective tumor-inhibiting amount of a member selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole and the therapeutically active acid addition salts thereof in a pharmaceutical carrier.

4. A process of aiding regression palliation of breast cancer which comprises the systemic administration to human or animal subjects hosting breast cancer of an effective antineoplastic amount of a member selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole and the therapeutically active acid addition salts thereof in a pharmaceutical carrier.

5. A process of aiding regression and palliation of breast cancer which comprises the systemic administration to a human hosting breast cancer of from about 1 to about 5 mg/kg body weight of the host of a member selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo-[2,1-b]thiazole and the therapeutically active acid addition salts thereof.

6. A process of aiding regression and palliation of lung cancer which comprises the systemic administration to human or animal subjects hosting lung cancer of an effective antineoplastic amount of a member selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole and the therapeutically active acid addition salts thereof in a pharmaceutical carrier.

7. A process of aiding regression and palliation of lung cancer which comprises the systemic administration to a human hosting lung cancer of from about 1 to about 5 mg/kg body weight of the host of a member selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole and the therapeutically active acid addition salts thereof.

8. A process of aiding regression and palliation of malignant melanoma which comprises the systemic administration to human or animal subjects hosting malignant melanoma of an effective antineoplastic amount of a member from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo[1,2-b]thiazole and the therapeutically active acid addition salts thereof in a pharmaceutical carrier.

9. A process of aiding regression and palliation of malignant melanoma which comprises the systemic administration to a human hosting malignant melanoma of from about 1 to about 5 mg/kg body weight of the host member selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole and the therapeutically active acid addition salts thereof.

10. A process of aiding regression and palliation of colorectal cancer which comprises the systemic administration to human or animal subject hosting colorectal cancer of an effective antineoplastic amount of a member selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo-[2,1-b]thiazole and the therapeutically active acid addition salts thereof in a pharmaceutical carrier.

11. A process of aiding regression and palliation of colorectal cancer which comprises the systemic administration to a human hosting colorectal cancer of from about 1 to about 5 mg/kg body weight of the host of a member selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo-[2,1-b]thiazole and the therapeutically active acid addition salts thereof.

12. A process of aiding regression and palliation of multiple myeloma which comprises the systemic administration to human or animal subjects hosting multiple myeloma of an effective antineoplastic amount of a memeber selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo-[2,1-b]thiazole and the therapeutically active acid addition salts thereof in a pharmaceutical carrier.

13. A process of aiding regression and palliation of multiple myeloma which comprises the systemic administration to a human hosting multiple myeloma of from about 1 to about 5 mg/kg body weight of the host of a member selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo-[2,1-b]thiazole and the therapeutcially active acid addition salts thereof.

14. A process of aiding regression and palliation of head and neck cancer which comprises the systemic administration to human or animal subjects hosting head and neck cancer of an effective antineoplastic amount of a member selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo-[2,1-b]thiazole and the therapeutically active acid addition salts thereof in a pharmaceutical carrier.

15. A process of aiding regression and palliation of head and neck cancer which comprises the systemic administration to a human hosting head and neck cancer of from about 1 to about 5 mg/kg body weight of the host of a member selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo-[2,1-b]thiazole and the therapeutically active acid addition salts thereof.

16. A process of aiding regression and palliation of gastric cancer which comprises the systemic administration to human and animal subjects hosting gastric cancer of an effective antineoplastic amount of a member selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo-[2,1-b]thiazole and the therapeutically active acid addition salts thereof in a pharmaceutical carrier.

17. A process of aiding regression and palliation of gastric cancer which comprises the systemic administration to a human hosting gastric cancer of from about 1 to about 5 mg/kg body weight of the host of a member selected from the group consisting of 2,3,5,6-tetrahydro-6-phenylimidazo-[2,1-b]thiazole and the therapeutically active acid addition salts thereof.

* * * * *

EXHIBIT B

Staple Here Only !

PRINTER'S TRIM LINE

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 4,584,305

DATED : April 22, 1985

INVENTOR(S) : Brugmans et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Claim 4, line 43 delete "regression palliation" and insert -- regerssion and palliation --.

Claim 8, line 21 delete "[1,2-b] and insert --[2,1-b] --.

Claim 12, line 50 delete "memeber" and insert -- member --.

MAILING ADDRESS OF SENDER: Geoffrey G. Dellenbaugh
Johnson & Johnson
One Johnson & Johnson Plaza
New Brunswick, NJ 08933-7003

PATENT NO. 4,584,305

No. of add'l. copies @ 30¢ per page

Johnson & Johnson

OFFICE OF
GENERAL COUNSEL

ONE JOHNSON & JOHNSON PLAZA
NEW BRUNSWICK, N.J. 08933 7003

June 10, 1986

The Hon. Commissioner of
Patents & Trademarks
Washington, D.C. 20231

Dear Sir:

Re: U.S. Patent No. 4,584,305
Serial No. 230,578
Our File: JAB 339

Dear Sir:

It is respectfully requested that a Certificate of Correction be issued to correct the error(s) as set forth in the attached form and in compliance with amended Rule 322.

Very truly yours,

Geoffrey G. Dellenbaughn
Attorney for Assignee
Reg. No. 26,864

GGD/sd
Attachment
One Johnson & Johnson Plaza
New Brunswick, NJ 08933-7003
(201) 524-5599

EXHIBIT C

Best Available Copy

SAB-339

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office
ADDRESS: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

PATENT NO.	PATENT DATE
4,584,305	4-22-86

PATENTEE
Josephus Brugmans et al

RECEIVED
AUG 25 1986
J&J PAT. DKT. SECTION

MAILING DATE
AUG 20 1986

Geoffrey D. Dellenbaugh
One Johnson and Johnson Plaza
New Brunswick, N.J. 08933-7003

NOTIFICATION OF APPROVAL IN-PART OF CERTIFICATE OF CORRECTION

The Certificate of Correction requested in the patent identified above has been APPROVED with the exceptions indicated below. The remaining errors will be corrected as requested. The Certificate, so modified, will be issued on SEP 30 1986

A. ☐ **THE CHANGES BELOW CANNOT BE INCLUDED IN THE CERTIFICATE SINCE THE REQUEST WAS FILED UNDER RULE 322.**

1. ☐ Column _____, line _____, is printed in accordance with the record.

(a) ☐ The change referred to was initialed and dated by applicant before execution of the application papers.

2. ☐ In column _____, line _____, the error resulted from applicant's failure to comply with Rule 121(a), in that the precise point of entry of the amendment was omitted.

3. ☐ In column _____, line _____, the alleged error is due to applicant's failure to comply with Rule 121(b), wherein provision is made for use of brackets, instead of parentheses, to cancel subject matter and for the use of interlineations to indicate new subject matter.

4. ☐ Omission of the priority data from the patent resulted from applicant's failure to fully comply with 35 U.S.C. 119, in that:

(a) ☐ The priority data was omitted from the oath, or declaration.

(b) ☐ The claim for priority was not included in the application papers.

(c) ☐ The certified copy of the foreign application was not filed.

5. ☐ The assignment data is printed in the patent in accordance with PTO 76-13b, submitted by applicant at time of payment of the base issue fee, as follows:

6. ☐ In column _____, line _____, the error arose because Rule 52(b) was not complied with. Consequently, words on top of certain pages were obliterated where those pages were placed in the file jacket, causing the Office to provide what appeared to be the proper words.

B. ☐ **THE REQUEST HAS BEEN CHANGED AS SHOWN BELOW TO COMPLY WITH THE RECORD:**

1. ☐ Since it is not normally the practice of the Office to reprint figures of the drawings, the following narrative description of Fig(s). _____ as suggested by the examiner, will be included:

(over)

Form PTOL-404 (rev. 10/80)

2. ☐ The error complained of in column _____, line _____, occurred in column _____, line _____, where the change's will be made.

3. ☐ The assignment will be shown in accordance with the Assignment Division records which read as follows:

4. ☒ The change requested in Claim 4, line 43 has been modified ~~by~~ to read. "regression pallation" should read -- regression and Palliation --

C. ☐ THE FOLLOWING CORRECTION(S) CANNOT BE INCLUDED IN THE CERTIFICATE FOR THE REASONS GIVEN BELOW:

1. ☐ The word _____, purported to be in column _____, line _____, cannot be found in the printed patent.

2. ☐ The alleged error in column _____, line _____, is an editing change made in accordance with the style of the Invention Patent Manual.

3. ☐ In column _____, line _____, the alleged error is in fact a change made by the examiner and considered to be in accordance with the permissible amendments enumerated in M.P.E.P. 1302.04.

4. ☐ In the title, it is the practice to exclude words such as "Improvements in", "New", etc., from the printed patent.

5. ☐ Comparison of the patent in column _____, line _____, with the corresponding location in the application file reveals that there is no discrepancy.

6. ☐ The records of the Assignment Division indicate that the patent has been printed in accordance with the assignment records at the time of issuance.

7. ☐ The numbering of the claims in the printed patent is in accordance with the renumbering of dependent claims by the examiner as described in M.P.E.P. 608.01(n).

8. ☐ The alleged error in column _____, line _____, is a change made in an Examiner's Amendment at time of allowance. Since no error is involved and since applicant filed no objection prior to payment of the base issue fee, the requested change will not be included in the Certificate.

9. ☐ The error complained of in column _____, line _____ cannot be corrected since the Group Director reports the following:

D. ☐ OTHER

Mary M. Allen
Supervisor, Certificates of Correction Branch

This decision is rendered pursuant to authority delegated by the Solicitor under authority delegated to him by the Commissioner of Patents and Trademarks.

Form PTOL-404 (rev. 10/80)

EXHIBIT D

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D. C. 20231

JAB-33

RECEIVED
SEP 25 1989
J&J PAT. DKT. SECTION

LEONARD F. PRUSAK
JOHNSON & JOHNSON
ONE JOHNSON & JOHNSON PLAZA
NEW BRUNSWICK, NY 08903

DATE MAILED
09/20/89

080660

MAINTENANCE FEE STATEMENT

The data shown below is from the records of the Patent and Trademark Office. If the maintenance fees and any necessary surcharges have been timely paid for the patents listed below, the notation "PAID" will appear in column 10, "status" below.

If a maintenance fee payment is defective, the reason is indicated by code in column 10, "status" below. An explanation of the codes appears on the reverse of the Maintenance Fee Statement. TIMELY CORRECTION IS REQUIRED IN ORDER TO AVOID EXPIRATION OF THE PATENT. NOTE 37 CFR 1.377. THE PAYMENT(S) WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION. IF PAYMENT OR CORRECTION IS SUBMITTED DURING THE GRACE PERIOD, A SURCHARGE IS ALSO REQUIRED. NOTE 37 CFR 1.20(k) and (l).

If the statement of small entity status is defective the reason is indicated below in column 10 for the related patent number. THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.

ITM NBR	PATENT NUMBER	FEE CDE	FEE AMOUNT	SUR CHARGE	SERIAL NUMBER	PATENT DATE	FILE DATE	PAY YR	SML ENT	
1	4,584,305	170	395	----	06/330,570	04/22/86	02/02 01	04	NO	P

If the "status" column for a patent number listed above does not indicate "PAID" a code or an asterisk (*) will appear in the "status" column. Where an asterisk (*) appears, the codes are set out below by the related item number. An explanation of the codes indicated in the "status" column and as set out below by the related item number appears on the reverse of the maintenance fee statement.

Copy for assignee
Date: 9/26/89
Signe

DIRECT THE RESPONSE TOGETHER WITH ANY QUESTIONS ABOUT THIS NOTICE TO:

MAINTENANCE FEE STATEMENT
STATUS CODES AND DEFINITIONS

CODE	DEFINITION
	IN REGARD TO THE MAINTENANCE FEE PAYMENT(S)
F160	The maintenance fee has already been paid. A refund of the payment has been scheduled to be sent to the fee address of record.
F161	The maintenance fee payment will not be accepted because it has been tendered too early. See 37 CFR 1.362. A refund of the payment has been scheduled.
F162	The maintenance fee payment does not properly identify the patent for which payment is to be made in accordance with 37 CFR 1.366(c). Either the U. S. application serial number or the patent number has been omitted. Both numbers are necessary to ensure proper crediting of the maintenance fee to the desired patent.
F163	The maintenance fee payment based upon certificate of mailing procedures is untimely, since it is not in compliance with the requirements of 37 CFR 1.8.
F164	The maintenance fee payment based upon "Express Mail" procedures is untimely since it is not in compliance with the requirements of 37 CFR 1.10.
F165	The maintenance fee and surcharge payment are not accepted because they have been submitted with the payment of fees for other purposes. See 37 CFR 1.366(e). A refund of the payment has been scheduled.
F166	The maintenance fee payment is not accepted because it is not immediately negotiable in the United States for the full payment of the required fee. Payment should be made in U. S. specie, Treasury notes, national bank notes, post office money orders or by certified check. See 37 CFR 1.23. The payment is returned herewith.
F167	The check or deposit account authorization is not accepted because it is unsigned. It is returned herewith.
F168	The payment received or the balance in the deposit account authorized for payment is insufficient to cover payment of the maintenance fee and surcharge, if any. Any payments accepted have been applied in accordance with the provisions of 37 CFR 1.366(e).
F169	The payment is in excess of the amount required. A refund has been scheduled.
	IN REGARD TO THE STATEMENT OF SMALL ENTITY STATUS
E180	A signature to the small entity statement is omitted.
E181	A small entity statement from each joint inventor has not been received.
E182	A small entity statement from the assignee or licensee has not been received.
E183	The requirements for filing as an independent inventor have not been met. See 37 CFR 1.9(c).
E 184	The requirements for filing as a small business concern have not been met. See 37 CFR 1.9(d).
E185	The requirements for filing as a nonprofit organization have not been met. See 37 CFR 1.9(e).
E186	The small entity statement was not verified by an oath or a declaration.

OMB Approved NO 0651-0016

FORM PTO-1536
(10-85)

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

MAINTENANCE FEE TRANSMITTAL FORM

Address: Commissioner of Patents and Trademarks
Box M. Fee
Washington, D.C. 20231

REEL 222 FRAME 436

ENTERED

Enclosed herewith is the payment of the maintenance fee(s) for the listed patent(s).

1. ☐ A check for the amount of $ _____ for the full payment of the maintenance fee(s) and any necessary surcharge on the following patents is enclosed.
2. XX ☐ The Commissioner is hereby authorized to charge $ 245.00 to cover the payment of the fee(s) indicated below to Deposit Account No 10-750
3. XX ☐ The Commissioner is hereby authorized to charge any deficiency in the payment of the required fee(s) or credit any overpayment to Deposit Account No. 10-750

*Information required by 37 CFR 1.366(c)(columns 1 & 5). *Information requested under 37 CFR 1.366(d)* (columns 2-4 & 6-9)

Item	Patent Number* 1	Fee Code 2	Maintenance Fee Amount 3	Surcharge Amount 4	U.S. Serial Number* 5 [06/555/555]	Patent Date 6 mm/dd/yy	Application Filing Date 7 mm/dd/yy	Payment Year 8	Small Entity? 9
1	4,584,305	170	$245.00		06/230,578	4/22/86	2/2/81	4	
2									
3									
4									
5									
6									
7									
8									
	Sub-totals—Columns 3 & 4		$245.00	-0-					
	Total Payment		$245.00						

Use additional sheets for listing additional patents.

P 30391 09/19/89 4584305 10-0750 030 170 245.00CH

(For Office Accounting Use Only)

Respectfully submitted

Charles J. Metz

(Payor's name)

(Payor number, if assigned)

(Payor's Telephone Number)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to "Commissioner of Patents and Trademarks, Box M. Fee, Washington, D.C. 20231" on _____.

Name of person signing _____

Signature _____

Note All correspondence will be forwarded to the "Fee Address" or the "Correspondence Address" if no "Fee Address" has been provided 37 CFR 1.363

37 CFR 1.20 Maintenance fees	Fee	Codes
(e) For maintaining an original or reissue patent, except a design or plant (See P.L. 98-622) patent, based on an application filed on or after December 12,1980 and before August 27, 1982, in force beyond 4 years, the fee is due by three years and six months after the original grant	$225.00	170
(f) For maintaining an original or reissue patent, except a design or plant (See P.L. 98-622) patent, based on an application filed on or after December 12,1980, and before August 27, 1982, in force beyond 8 years, the fee is due by seven years and six months after the original grant	$445.00	171
(g) For maintaining an original or reissue patent, except a design or plant (See P.L. 98-622) patent, based on an application filed on or after December 12, 1980, and before August 27, 1982, in force beyond 12 years, the fee is due by eleven years and six months after the original grant	$670.00	172
(h) For maintaining an original or reissue patent, except a design or plant patent, based on an application filed on or after August 27, 1982 in force beyond 4 years; the fee is due by three years and six months after the original grant.		
By a small entity (§ 1.9(f))	$225.00	273
By other than a small entity	$450.00	173
(i) For maintaining an original or reissue patent, except a design or plant patent, based on an application filed on or after August 27, 1982 in force beyond 8 years; the fee is due by seven years and six months after the original grant.		
By a small entity (§ 1.9(f))	$445.00	274
By other than a small entity	$890.00	174
(j) For maintaining an original or reissue patent, except a design or plant patent, based on an application filed on or after August 27, 1982 in force beyond 12 years, the fee is due by eleven years and six months after the original grant.		
By a small entity (§ 1.9(f))	$670.00	275
By other than a small entity	$1340.00	175

Surcharges	Fee	Codes
(k) Surcharge for paying a maintenance fee during the 6-month grace period following the expiration of three years and six months, seven years and six months, and eleven years and six months after the date of the original grant of a patent based on an application filed on or after December 12, 1980 and before August 27, 1982	$110.00	176
(l) Surcharge for paying a maintenance fee during the 6-month grace period following expiration of three years and six months, seven years and six months, and eleven years and six months after the date of the original grant of a patent based on an application filed on or after August 27, 1982.		
By a small entity (§ 1.9(f))	$55.00	277
By other than a small entity	$110.00	177
(m) Surcharge for accepting a maintenance fee after expiration of a patent for non-timely payment of a maintenance fee (See P.L. 98-622) where the delay in payment is shown to the satisfaction of the Commissioner to have been unavoidable	$500.00	178

EXPLANATION OF INFORMATION REQUESTED

1. **Patent Number**—The Patent Number of the patent on which a maintenance fee is being paid. Required by 37 CFR 1.366. For a reissue patent, the original patent number, patent date and filing date should also be included between parentheses in the same box as the reissue patent data giving the reissue patent number, reissue patent date, and reissue application filing date respectively.

2. **Fee Code**—Patent and Trademark Office Fee Code listed above. Used by Office to credit fees to appropriate fee category.

3. **Maintenance Fee Amount**—Amount listed in 37 CFR 1.20 for maintenance fee being paid.

4. **Surcharge Amount**—If the maintenance fee is paid after the due date, a surcharge is required in the amount indicated in 37 CFR 1.20 (k) - (m)

5. **U.S. Serial Number**—The United States Serial Number of the United States application for patent on which a maintenance fee is being paid Required under 37 CFR 1.366(c). The two digit series code should be included as part of the serial number. All applications filed after 1979 have Series Code 06/.

6. **Patent date**—The issue date of the patent on which a maintenance fee is being paid.

7. **Application Filing Date**—The United States filing date as defined in 37 CFR 1.362(c).

8. **Payment Year**—An indication should be made as to whether the maintenance fee being paid is that required to be paid by 4, 8, or 12 years after the patent date to prevent expiration of the patent.

9. **Small Entity**—Maintenance fees paid on patents based on applications filed on or after August 27, 1982 should also indicate by a "Yes" or "No" whether small entity status is being claimed.

EXHIBIT E

Surgical Adjuvant Therapy of Large-Bowel Carcinoma: An Evaluation of Levamisole and the Combination of Levamisole and Fluorouracil

By John A. Laurie, Charles G. Moertel, Thomas R. Fleming, Harry S. Wieand, John E. Leigh, Joseph Rubin, Greg W. McCormack, James B. Gerstner, James E. Krook, James Malliard, Donald I. Twito, Roscoe F. Morton, Loren K. Tschetter, and John F. Barlow for the North Central Cancer Treatment Group and the Mayo Clinic

A total of 401 eligible patients with resected stages B and C colorectal carcinoma were randomly assigned to no-further therapy or to adjuvant treatment with either levamisole alone, 150 mg/d for 3 days every 2 weeks for 1 year, or levamisole plus fluorouracil (5-FU), 450 mg/m²/d intravenously (IV) for 5 days and beginning at 28 days, 450 mg/m² weekly for 1 year. Levamisole plus 5-FU, and to a lesser extent levamisole alone, reduced cancer recurrence in comparison with no adjuvant therapy. These differences, after correction for imbalances in prognostic variables, were only suggestive for levamisole alone ($P = .05$) but quite significant for levamisole plus 5-FU ($P = .003$). Whereas both treatment regimens were associated with overall improvements in survival, these improvements reached borderline significance only for stage C patients treated with levamisole plus 5-FU ($P = .03$). Therapy was clinically tolerable with either regimen and severe toxicity was uncommon. These promising results have led to a large national intergroup confirmatory trial currently in progress.

J Clin Oncol 7:1447-1456. © 1989 by American Society of Clinical Oncology.

CARCINOMA of the large bowel, the colon, and the rectum is one of the most common malignant diseases encountered in Western civilization and one of the most frequent causes of cancer death. Although the great majority of these patients are discovered at a stage of disease where surgical resection with curative intent is possible, national end-result statistics still show that almost half of the patients afflicted with large-bowel cancer will die of it. Early diagnosis and prevention are laudable future goals, but for the patient today and in the years immediately ahead the most realistic hope would seem to lie in attempts to enhance the effectiveness of surgical therapy. Although historically controlled studies have predictably been positive, all past randomized studies of colon cancer surgical adjuvant therapy compared with untreated controls have either been clearly negative or, at best, highly equivocal in results. Notable past chemotherapy failures have included thiotepa,[1] fluorouracil (5-FU) by a variety of doses and schedules,[1] and 5-fluoro-2-deoxyuridine (5-FUDR).[1] Even when results of a selected group of 5-FU studies involving a total of 3,182 patients were summated in a retrospective meta-analysis, any therapeutic advantage was, at best, marginal.[2] This led the authors to conclude that their meta-analysis "provides only moderate evidence that adjuvant chemotherapy may provide an overall survival benefit but strong evidence that such benefit, if it indeed exists, would likely be small: the effect of prolonged fluorouracil-containing chemotherapy results in a five-year survival benefit of less than 5%." A recent study of the combination of 5-FU, lomustine (methyl CCNU), and vincristine reported a survival advantage that approached, but did not reach, the traditional $P < .05$ level of significance.[3] These results have not been confirmed and any true therapeutic benefit must be questioned because three

From the Grand Forks Clinic, Ltd, Grand Forks, ND; Mayo Clinic, Rochester, MN; Fred Hutchinson Cancer Research Center, Seattle, WA; Fargo Clinic Community Clinical Oncology Program, Fargo, ND; Illinois Oncology Research Association Community Clinical Oncology Program, Peoria, IL; The Duluth Clinic Community Clinical Oncology Program, Duluth, MN; Creighton University, Omaha, NE; Billings Clinic, Billings, MT; Iowa Oncology Research Association Community Clinical Oncology Program, Des Moines, IA; Sioux Falls Community Cancer Consortium Community Clinical Oncology Program, Sioux Falls, SD; and the Clinical Laboratory of the Black Hills, Rapid City, SD.

Submitted February 16, 1989; accepted May 26, 1989.

Supported in part by Public Health Service Grants No. CA-25224, CA-15083, CA-35101, CA-35103, CA-35113, CA-35269, and CA-37417 from the National Cancer Institute, Department of Health and Human Services.

Address reprint requests to Charles G. Moertel, MD, Mayo Clinic, Rochester, MN 55905.

0732-183X/89/0710-0021$03.00

other studies have failed to demonstrate a significant advantage for 5-FU plus methyl CCNU over untreated controls,[4] and because a large study comparing 5-FU/methyl CCNU with 5-FU alone produced essentially identical survival experiences.[5] Similarly unconvincing have been a smattering of immunotherapy attempts such as bacillus Calmette-Guerin (BCG),[3,6] MER-BCG,[7] and C parvum.[8] However, there have been two positive trials. The first involved portal-vein infusion of 5-FU,[9] a method that is currently the subject of confirmatory studies. The second was a small trial conducted by Verhaegen et al in which patients with surgically treated, large-bowel cancer were assigned after surgery to no-further treatment or to levamisole therapy.[10] These investigators reported a striking and statistically significant survival advantage for the levamisole-treated group.

Levamisole has been widely used as an antihelminthic drug for over 20 years. It attracted interest for possible cancer treatment in the 1970s when it was found to have immunostimulatory activity.[11,12] In some animal tumor models it showed antineoplastic effects that seemed more striking against metastasis. There was also impressive activity in some animal models simulating the surgical adjuvant setting. In view of these findings, as well as the mild toxicity of the drug, a number of human therapeutic trials were initiated, several of which have claimed positive results. We felt the interesting result in colorectal cancer reported by Verhaegen et al[10] merited a confirmatory attempt. In addition, on an empiric basis, we elected to study a regimen of 5-FU plus levamisole. For the 5-FU regimen in this combination we chose a 5-day intensive course followed by weekly maintenance since among the many negative 5-FU colon surgical adjuvant trials, a similar schedule used by the Central Oncology Group had shown a suggestive but not significant therapeutic advantage for 5-FU ($P = .21$).[13] It was our judgment that the inclusion of an untreated control arm was mandatory. To avoid the data quality problems produced by patient refusal of treatment assignment, we also elected to obtain patient informed consent prior to randomization. We presented the preliminary results of this trial in 1986,[14] and this is our final report.

METHODS

Patient Selection

All patients were required to have had a potentially curative resection of a histologically confirmed adenocarcinoma of the colon or rectum. There could be neither gross nor microscopic evidence of residual disease, and it was required that the margins of resection be demonstrated free of tumor. It was also required that the resected specimen show one of the following indicators of poor prognosis: invasion of serosa or pericolonic fat, invasion of adjacent organs by direct extension, or metastasis to regional lymph nodes. No patient could have had any prior radiation therapy to the lumbar spine or pelvis or any prior 5-FU therapy. It was further required that the patient reliably tolerate oral medications and have a WBC count of 4000/μL or greater and a platelet count of 130,000/μL or greater. Patients were ineligible if they had had any other malignant disease within the previous 5 years except for superficial squamous or basal cell carcinoma of the skin or in situ carcinoma of the cervix. Patients were also ineligible if they had any evidence of distant metastasis or any regional metastasis that could not be resected en bloc with the primary lesion. To ensure eligibility, surgery and pathology reports were reviewed on each patient and slides of tissue were reviewed by the North Central Cancer Treatment Group (NCCTG) Pathology Committee. Patients were allowed entry in the study as soon during the postoperative course as they were able to reliably tolerate oral medication, but they could not enter any later than 5 weeks after surgery.

Stratification and Randomization Procedures

A signed informed consent was obtained prior to study entry. Patients were then stratified according to stage defined as: (1) invasion into or through serosa or into pericolonic or perirectal fat, or involving adjacent organs by direct extension; no lymph node metastasis (stages B2 and B3); (2) metastasis involving one to four regional lymph nodes (stage C1) with the primary tumor not invading into or through serosa; (3) metastasis involving one to four regional lymph nodes (stage C1) with the primary tumor extending through bowel wall and invading serosa, pericolonic or perirectal fat, or adjacent organs; and (4) metastasis involving more than four regional lymph nodes (stage C2). Patients were also stratified according to the following anatomic location criteria: (1) cecum, ascending or transverse colon; (2) descending, sigmoid, or rectosigmoid colon; and (3) rectum with all tumor below peritoneal reflection. Following stratification, patients were randomized to the following study arms: (1) follow-up without adjuvant therapy; (2) levamisole therapy; or (3) levamisole plus 5-FU therapy. For patients assigned to active treatment, it was required that this treatment be initiated within 6 days of randomization.

Protocol Management

Within 72 hours prior to randomization it was required that the patient have a medical history, a physical examination, a hematology grouping including hemoglobin, leukocyte count, platelet count, and differential count, a blood chemistry panel, and a chest x-ray if one was not obtained preopera-

tively. Management after randomization is described below according to treatment arm.

No adjuvant therapy. Patients assigned to the control arm were observed after surgery with no planned treatment. During the first year they were reevaluated every 8 weeks, during the second year every 4 months, and following every 6 months for a total of 5 years. These evaluations consisted of an interim history and physical examination, blood counts, a blood chemistry panel, and a chest x-ray. In addition, this group of patients had either a proctoscopic examination and colon x-ray (barium enema) or a colonoscopic examination every 6 months. Follow-up after 5 years was continued but without formal protocol requirements for evaluation procedures.

Levamisole alone. Patients randomized to this arm had periodic evaluations at the same time as patients randomized to the no-adjuvant-therapy arm. In addition, they received levamisole, 50 mg, every 8 hours over 3 days, which was repeated every 2 weeks for 1 year. During the period of treatment, hematology groups and blood chemistry groups were repeated every 4 weeks.

Levamisole plus 5-FU. Patients randomized to this arm had periodic evaluations at the same time as patients randomized to the no-adjuvant-therapy arm. These patients were given levamisole at the same dose and schedule as described above for patients randomized to levamisole alone. In addition, they received 5-FU at a dose of 450 mg/m^2/d by rapid intravenous (IV) injection for 5 consecutive days. Beginning on day 28 they were initiated on weekly injections of 5-FU at a dose of 450 mg/m^2. 5-FU therapy was also administered over a total period of 1 year. Leukocyte counts were obtained before each weekly dose of 5-FU. If the patient experienced stomatitis, diarrhea, or leukopenia during weekly 5-FU administration, 5-FU administration was deferred until these side effects subsided. If these side effects were moderate to severe in intensity, 5-FU was resumed at a 20% reduction in dose.

Statistical Methods

The protocol was designed to assure that there would be a 75% probability of detecting a 50% increase in survival for a pairwise comparison of treatments using a one-sided log-rank test at significance level of 0.05. Survival, time to recurrence, and recurrence rates were identified as primary end points.

Statistical analyses were carried out using Statistical Application System (SAS) procedures.[15] The survival curves were generated using the Kaplan-Meier method.[16] The log-rank statistic[17] was used for the comparison of survival distributions. Since our study was originally designed for all *P* values for treatment comparisons versus control to be one-sided, all results will be presented in this manner. We chose a one-sided test because we had a "standard therapy" untreated control arm, because our only objective and interest was to improve therapeutic results in comparison with control, and because other studies with levamisole alone as surgical adjuvant therapy or in combination with 5-FU in advanced disease made it unlikely that either of our treatment arms would detract from recurrence-free time or survival. We recognize and respect an alternative view that two-sided tests may be more appropriate under these circumstances, and they certainly represent a more conservative approach. For our primary end results (overall intervals to progression and survivals) we have therefore provided both one-sided and two-sided *P* values. The Cox proportional hazards model[18] was used to obtain the ratios of relapse and survival rates and for all multivariate analyses. A backward regression was used to find the most significant factors, variables being eliminated based on the maximum partial likelihood estimate (MLE) statistics. To adjust for covariates when evaluating treatments, we kept treatment in the model and used the backward regression for other covariates, keeping covariates whose MLE statistics satisfied $P < .05$.

RESULTS

A total of 408 patients were entered in this study. Seven patients (1.7%) were declared ineligible, primarily because of inappropriate stage of disease. Because eligibility would not be biased by treatment assignment, these patients were excluded from the analyses to follow. Among the 401 eligible patients there were four (1%) who refused their randomized assignment. Because this decision could be biased by treatment assignment, these patients are included in all of the analyses to follow. We have conducted separate analyses including the ineligible patients or excluding the cancelled patients. These analyses will not be presented since, as is evident by their small numbers, data from these patients had no substantive influence on results.

Patient Characteristics

Characteristics of our 401 eligible patients are displayed according to study arm assignment in Table 1. Overall, it can be seen that there was essentially an equal sex distribution. Mean and median age for all patients was 61 years with a range from 22 to 83 years. There is a disproportionately small representation of tumors primary to the rectum, no doubt related to the fact that the NCCTG developed an alternative protocol for primary rectal carcinoma approximately 1 year after this protocol was opened. Two thirds of all patients had nodal metastasis (stage C), and the great majority of these had from one to four nodes involved (stage C1). A total of only 26 patients had nodal involvement without invasion of serosa or pericolonic fat. Most patients had tumors that were either well or moderately differentiated and less than 20% had a highly anaplastic histology. As is evident in Table 1, patient characteristics are remarkably well distributed between the three study arms. The only imbalances, and these of a minor degree, are a slightly

Table 1. Patient Characteristics According To Treatment Arm

Characteristics	Surgery Only (%) (n = 135)	Levamisole Alone (%) (n = 130)	Levamisole Plus 5-FU (%) (n = 136)
Male	50	49	51
Age			
Median	61	61	61
Range	27-81	23-83	22-82
Stage			
B2 and B3	36	35	33
C1 (1-4 nodes, no serosal invasion)	8	8	10
C1 (1-4 nodes, serosal invasion)	39	41	40
C2 (> 4 nodes)	17	17	17
Anatomic site, primary			
Ascending and transverse	47	48	44
Descending and sigmoid	47	45	47
Rectum	6	6	9
Grade of anaplasia*			
1-2	81	83	81
3-4	19	17	19
Performance score†			
0	76	79	68
1	20	14	27
2-3	4	6	5

*Broders' grades 1, well-differentiated to 4, highly undifferentiated.

†Eastern Cooperative Oncology Group score 0, fully active to 4, totally disabled.

larger proportion of patients with nodal involvement, with a rectal primary, and with impaired performance on the levamisole/5-FU arm when compared with the control arm.

Therapeutic Results

At present, this study is very mature. The median follow-up is 7 years and 9 months, and the minimum follow-up is just over 4 years. No patient has been lost to follow-up.

Tumor Recurrence

At the time of this writing, 191 patients have experienced recurrent colorectal carcinoma, and it is estimated that nearly 100% of all anticipated recurrences have been documented. The estimated overall reduction in recurrence rate (Cox proportional hazards model) for levamisole alone is 27% with a 90% confidence interval from 2% to 45%, and for levamisole plus 5-FU it is 31% with a confidence interval from 8% to 48%. Plots of recurrence-free intervals for all eligible patients are displayed in Fig 1. The levamisole plus 5-FU

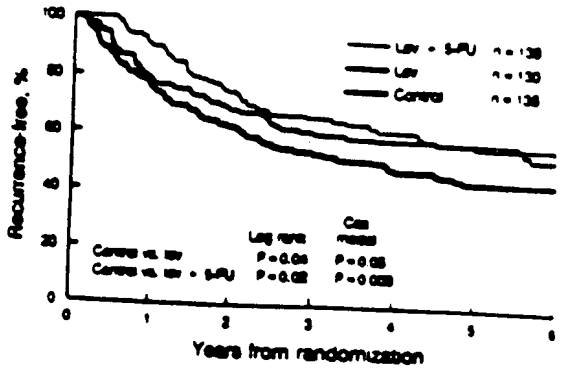


Fig 1. Recurrence-free interval, all patients.

combination shows a clear advantage over control, whereas the advantage is borderline for levamisole alone (respective one-sided *P* values, .02 and .04; two-sided, .04 and .08). It is apparent that the combination provides not only fewer recurrences but also a delay for the recurrences that are observed. Figures 2 and 3 demonstrate similar relationships between treatment arms when the patient populations are divided into subsets according to stage. The advantage for levamisole plus 5-FU is significant only in stage C. In exploring other subsets we found the most favorable treatment advantages to be in primary tumors of the ascending and transverse colon for both levamisole and levamisole plus 5-FU; in females, for levamisole plus 5-FU; in lesions of low to moderate degrees of anaplasia, for 5-FU plus levamisole; and in the less than 65-year age group, for levamisole plus 5-FU. However, these subset analyses must be interpreted with great caution. In no case did we find a significant interaction of treatment with a covariate.

Table 2 displays a number of potential prognostic determinants for recurrence. Tumor stage,

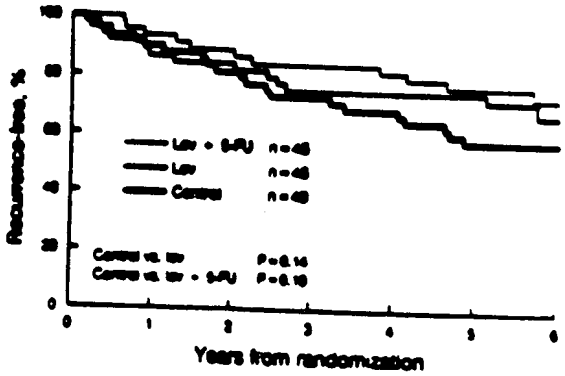


Fig 2. Recurrence-free interval, stage B.

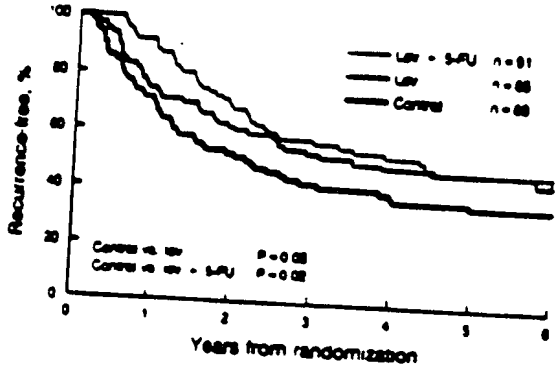


Fig 3. Recurrence-free interval, stage C.

primary location, and grade of anaplasia were strongly determinant and performance score was of borderline significance, whereas age and sex played no important role. Using a step-wise proportional hazard model to correct for the influence of prognostic variables, levamisole again showed a borderline advantage over control with a one-sided P value of .05 (two-sided, .10), whereas levamisole plus 5-FU showed a very significant advantage with a one-sided P value of .003 (two-sided, .006). In the multivariate analysis, stage, location, grade, and performance score were all significant (two-sided, $P < .05$). The increased significance associated with levamisole and 5-FU in the multivariate analysis appears to be due primarily to the unfavorable imbalance in performance score and, to a lesser extent, to the imbalance in the number of rectal patients.

In the analyses above, patients who died without recurrence were censored. Analyses were also performed in which death without recurrence was considered an event (ie, progression-free survival). These analyses produced comparable results to those recorded above.

Table 2. Prognostic Determinants For Recurrence

Determinant	No. of Patients	Percentage Recurring by 5 Years	P*
Sex			
Male	201	49	.19
Female	200	44	
Age			
1-64 yr	253	48	.21
≥ 65 yr	148	43	
Location, primary			
Ascending and transverse	186	39	.0013
Descending and sigmoid	187	49	
Rectum	28	75	
Grade of anaplasia†			
1-2	315	43	.0010
3-4	83	61	
Stage			
B2 and B3	139	30	.0000
C1 (1-4 nodes, no serosal invasion)	35	32	
C1 (1-4 nodes, serosal invasion)	159	54	
C2 (> 4 nodes)	68	69	
Performance score			
0	297	44	.13
1-3	104	52	

*Two-sided log-rank.

†Three patients had an unknown tumor grade.

Survival

At the time of this writing, 195 patients have died and an estimated 95% of the deaths within 5 years of study entry have been documented. There have been 21 deaths without evidence of recurrence: five on the control arm, eight on levamisole alone, and eight on levamisole plus 5-FU. There are 17 patients who have had known recurrence but are still alive: nine on the control arm, five on levamisole alone, and three on levamisole plus 5-FU. These data make it likely that any survival advantage for levamisole 5-FU will increase in the future.

Plots of survival for all eligible patients are displayed in Fig 4. Perhaps because of disproportionate deaths without recurrence and disproportionate recurrences without deaths, these differences are not as striking as differences in recurrence rates (one-sided P value for levamisole alone, .13 and for 5-FU/levamisole, .22; two-sided, .26 and .44). The estimated overall reduction in death rate (Cox proportional hazards model) on levamisole alone is 18% with a

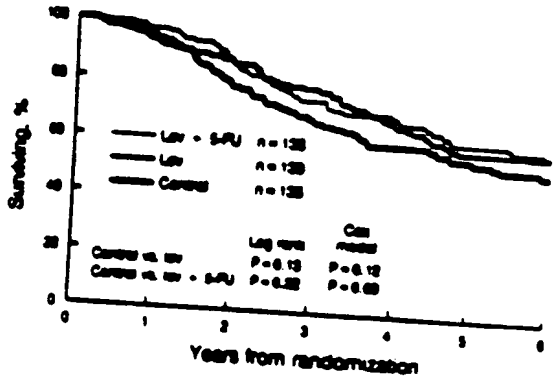


Fig 4. Survival, all patients.

90% confidence level of 39% to −10%, and for levamisole plus 5-FU, 13%, with a confidence level of 34% to −16%.

Figure 5 shows little difference in survival times for stage B patients. Differences are more striking among stage C patients (Fig 6), with a stronger advantage for levamisole plus 5-FU. No meaningful differences were found in exploration of other subsets.

Table 3 displays a number of potential prognostic determinants for survival. As was true for recurrence, stage and primary location and grade of anaplasia had strong and independent predictive values for survival, whereas performance score was of borderline significance. After correction for the influence of prognostic variables, both levamisole and levamisole plus 5-FU showed only suggestive treatment advantages (one-sided P values, .12 and .09, respectively; two-sided, .24 and .18). However, within the stage C subset, levamisole/5-FU provided an advantage over control after correction at the one-sided $P = .03$ level (two-sided, $P = .06$). As in recurrence, the increased significance associated with levamisole and 5-FU seems to be attributable primarily to the imbalance in performance score and rectal site of primary.

Toxicity

Toxic reactions according to treatment arm are presented in Tables 4 and 5. Reactions to levamisole alone were infrequent, consisting primarily of mild nausea, mild diarrhea, and mild leukopenia. The lowest leukocyte count recorded was 2,000/μL and the lowest platelet count, 40,000/μL. One patient experienced severe dermatitis requiring discontinuance of therapy. A frequent minor symptom was a metallic taste in the mouth.

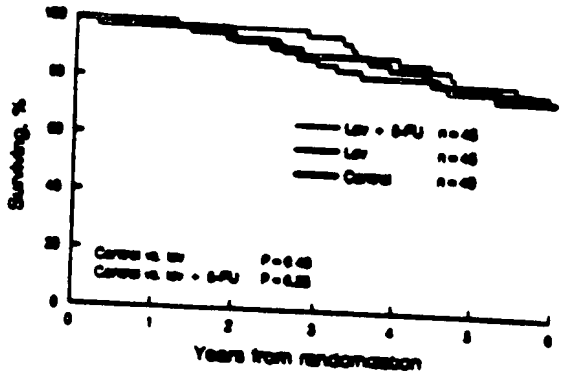


Fig 5. Survival, stage B.

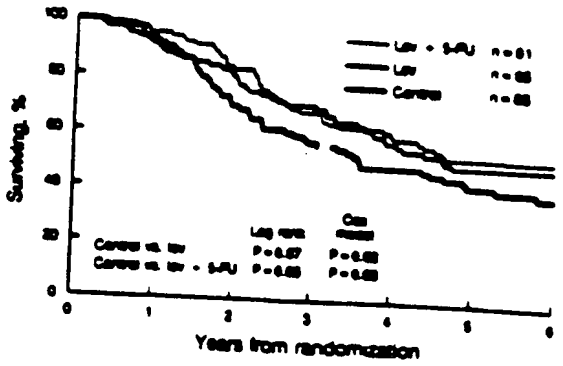


Fig 6. Survival, stage C.

Toxic reactions to the combination arm were consistent with what might be anticipated with 5-FU alone with the exception of perhaps a bit more hematologic depression in the weekly maintenance phase. Five patients (4%) experienced leukopenia of 1,000/μL or less, and three patients experienced sepsis. There were no treatment-related deaths.

Treatment Compliance

Treatment compliance, in the main, was excellent. Ninety-five percent of eligible patients ran-

Table 3. Prognostic Determinants for Survival

Determinant	No. of Eligible Patients	Percentage Surviving at 5 Years	P*
Sex			
Male	201	60	.84
Female	200	58	
Age			
1-64 yr	253	61	.19
≥ 65 yr	148	55	
Location, primary			
Ascending and transverse	186	63	.033
Descending and sigmoid	187	58	
Rectum	28	39	
Grade of anaplasia			
1-2	315	60	.023
3-4	83	52	
Stage			
B2 and B3	139	78	.0000
C1 (1-4 nodes, no serosal invasion)	38	63	
C1 (1-4 nodes, serosal invasion)	159	50	
C2 (> 4 nodes)	68	37	
Performance score			
0	297	61	.09
1-3	104	52	

*Two-sided log-rank.

Table 4. Toxicity—Nonhematologic

Toxic Reaction	Levamisole Alone (%) (n = 130)	Levamisole plus FU (%) (n = 133)
Nausea	18	68
Vomiting	5	23
Diarrhea	13	53
Stomatitis	2	28
Dermatitis	5	20
Alopecia	3	21

NOTE. Cancelled patients excluded.

domized to the levamisole-alone arm and 83% of those randomized to levamisole plus 5-FU were totally compliant, continuing therapy for 1 year or until required by protocol to discontinue therapy because of toxicity or recurrence. On the levamisole-alone arm seven patients withdrew from treatment before completion in the absence of recurrence or severe toxicity. On the 5-FU-plus-levamisole arm, three patients refused their treatment assignment and 20 patients withdrew from treatment before completion and in the absence of recurrence or severe toxicity. Seven patients assigned to levamisole alone had therapy discontinued because of toxicity or other medical reasons, as did 10 patients assigned to levamisole plus 5-FU. No levamisole-alone patient had therapy temporarily interrupted because of toxicity. On the levamisole-plus-5-FU arm 33 patients had a dosage reduction or temporary treatment interruption because of toxicity. Eight patients on the combination arm required discontinuance of levamisole but continued 5-FU alone, whereas six patients required discontinuance of 5-FU but continued levamisole alone.

Second Primary Malignant Diseases

Twenty patients have developed second primary cancers since entry into this study. Four of these were on the control arm, seven on levamisole-alone arm, and nine on levamisole-plus-5-FU arm. Seven patients developed a second primary colorectal cancer that was clearly separate from the previous line of anastomosis. The other second primaries were three gastric, two lung, and one each of bile duct, bladder, breast, esophagus, ovary, prostate, pancreas, and kidney. No patient has developed leukemia. The small increases in second primaries on the levamisole and levamisole-plus-5-FU arms are not statistically significant and are not likely to be clinically meaningful in view of the large number of controlled studies of levamisole conducted in other diseases without documentation of a similar finding.

Table 5. Toxicity—Hematologic

Toxic Reaction	Levamisole Alone (%) (n = 124)	Levamisole plus 5-FU Induction (%) (n = 128)	Levamisole plus 5-FU Maintenance (%) (n = 118)
Leukopenia			
< 4,000 ≥ 2,000	14	47	42
< 2,000	0	12	3
Thrombocytopenia			
< 130,000 ≥ 50,000	3	10	24
< 50,000	1	0	0

NOTE. Excludes cancelled patients and patients with inadequate counts recorded.

DISCUSSION

This study indicates the likelihood that adjuvant therapy with levamisole plus 5-FU substantially reduces the recurrence rate for patients with resected but poor-prognosis large-bowel cancer. It also indicates the possibility that a similar result might be obtained with levamisole alone. Either therapy is patient-tolerable, associated with a minimal risk, and could easily be adopted as standard practice. However, before regarding the results of this study as a major cancer treatment breakthough, several precautionary notes should be sounded. Whereas we have documented significant reductions in cancer recurrence, we have documented significant survival improvement in only our stage C subset. Survival is the only hard end point of a surgical adjuvant trial, and subset analyses are notorious for their misleading results. In addition, the past record of levamisole in human therapeutic trials indicates that positive results can be very fragile and evanescent.

Levamisole is a drug with a fascinating history. Globally, it has been an agent of singular importance, due to some extent to its use as an antihelminthic agent in humans in Third World countries, but particularly because of its effectiveness in treating nematode infections in farm animals where it has had a major effect on agricultural economy.[10] It has periodically been claimed to have effectiveness for a variety of therapeutically intractible, disabling, or life-threatening human diseases including rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis, chronic hepatitis, simple and genital herpes, Crohn's disease, ulcerative colitis, upper

JOBNAME: JCO October PAGE: 8 SESS: 15 OUTPUT: Mon Aug 21 1[illegible]:40 1989
/scr1/disk4/wbs/jcln/jclnoct/3[illegible]

respiratory infections, aphthous stomatitis, warts, and leprosy. However, essentially all of these claims for effectiveness have been disclaimed by later and more scientifically rigorous studies.

Levamisole has been extensively evaluated in cancer clinical trials, particularly in the minimal disease and surgical adjuvant settings. However, again evidence for therapeutic benefit has almost invariably failed the test of confirmation. For example, in postmenopausal, stage II breast cancer a randomized Finnish trial claimed that levamisole surgical-adjuvant therapy produced a highly significant improvement in survival ($P = .003$),[19] whereas a large trial from The Netherlands showed no benefit[20] and an even larger study by a Danish group showed levamisole to be associated with a less favorable survival than untreated controls.[21] As surgical adjuvant treatment for non–small-cell lung cancer, a randomized trial by Amery et al claimed that levamisole produced a significant survival advantage,[22] whereas the randomized trial by Van Houtte et al showed no survival advantage at all for levamisole,[23] and in stark contrast, the trials by Anthony et al[24] and Herskovic et al[25] actually showed the survival of levamisole-treated patients to be inferior to that of placebo-treated patients. In malignant melanoma a Canadian surgical adjuvant trial showed a significant advantage for levamisole over untreated controls.[26] However, in this same study when levamisole was combined with BCG, no advantage whatsoever was obtained and in a comparable melanoma adjuvant trial, Spittler et al found levamisole therapy to produce no advantage over placebo in either time to recurrence or survival.[27]

The same confusion of conflicting levamisole results is seen for colorectal carcinoma specifically. It begins in the basic laboratory setting where three animal model colorectal carcinoma surgical-adjuvant studies produced two results that were strongly positive[28,29] and one result that was decisively negative.[30] In human surgical adjuvant trials, the small Veterans trial showed a significant survival advantage for levamisole-treated patients. Our trial shows a borderline significant advantage in tumor recurrence but only a suggestion of advantage in survival. A small trial of the Western Cancer Study Group (only 26 patients on the control arm) was completely negative.[31] A large and much more convincing trial of the European Organization for Research and Treatment of Cancer (EORTC) compared levamisole with placebo therapy in 297 surgically treated colon cancer patients. They reported 5-year survival rates at 51% (SE, 5.4%) with levamisole versus 39% (SE, 5.9%) with placebo. The overall survival difference, however, was not significant ($P = 0.35$) and they concluded that the overall effect of therapy, if any, was likely to be small.

The most convincing evidence of meaningful therapeutic activity produced by our study is the combination of levamisole and 5-FU. Here we have a significant delay in tumor recurrence, a significant overall reduction of tumor recurrence, and a suggestion of improved survival that is particularly evident in our stage C subset. The validity of these observations is enhanced by the recent colon surgical adjuvant trial of Windle et al, who randomized patients between no treatment, 5-FU alone, and a levamisole/5-FU combination.[32] Although this study was very small (only 42 to 45 patients per treatment arm), it did demonstrate a highly significant survival advantage for patients treated with the levamisole/5-FU combination in comparison with both no treatment and 5-FU alone. However, it should be noted that there were very important differences between this study and ours. These differences include a large proportion of rectal cancer patients and considerable difference in regimen for both levamisole and 5-FU.

It is interesting to speculate why the addition of levamisole to 5-FU may be producing a positive colorectal cancer surgical adjuvant result in spite of the fact that results in this setting with levamisole alone must be considered equivocal, and numerous studies with 5-FU alone have failed to demonstrate a significant survival advantage. Certainly, the most immediate presumption is that levamisole is acting as an immunorestorative agent in patients who are immunosuppressed by both recent surgery and subsequent chemotherapy. There is a wealth of animal-model evidence, as well as some human evidence, that levamisole does have immunomodulatory activity[9,10,33] and that this activity is particularly exerted in the host with suppressed immune mechanisms. This effect is probably exerted through T-cell activation, augmentation of macrophage activity, and an increase in chemotactic response of polymorphonuclear cells and monocytes. In some animal tumor models levamisole

has clearly demonstrated an antineoplastic effect.[34] However, with all this it is disturbing that there has not been any consistent demonstration of antineoplastic activity in the human setting. An alternative postulate is that levamisole may be contributing to the activity of 5-FU in a manner that is entirely independent of immune modulation. Levamisole has a broad spectrum of additional pharmacologic activities. It has cholinergic properties, it possesses some mood-elevating effects in humans, and at high doses it acts as a convulsant.[34] It probably influences prostaglandin activity, it is a specific inhibitor of fumarate reductase in several nematodes, and it is a very potent inhibitor of mammalian alkaline phosphatases. In a number of animal tumor models the combinations of levamisole with cyclophosphamide or methyl CCNU or carmustine (BCNU) have produced striking improvements in animal survival when compared with any of these chemotherapeutic agents used alone.[35] It is of interest that in most of these animal tumor models levamisole alone has no effect whatsoever on survival. Whereas it is tempting to state that levamisole is acting as a biochemical modulator of 5-FU activity, attempts to demonstrate this effect clinically have predictably met with conflicting results, at least in the advanced disease setting. For a randomized comparison of 5-FU alone versus levamisole plus 5-FU, Borden et al gave a preliminary report suggesting a survival advantage for patients treated with the combination.[35] In contrast, a study by our group of a similar, but not identical, levamisole/5-FU combination produced a survival curve that completely overlapped that of 5-FU alone.[36] Finally, one could speculate that levamisole had no role at all and that our study simply demonstrates effectiveness of 5-FU alone as displayed by modern clinical trial methodology. Whereas the results of Windle et al speak against this,[32] the remote possibility cannot be denied.

Certainly, the checkered history of levamisole trials in human cancer speaks strongly against overinterpretation of the results of any single trial. Nevertheless, the results of our study are provocative and raise the hope that a step of progress may have been taken. Even though the results of Windle et al would appear to give confirmation,[32] this study involved only a relatively few patients and their levamisole/5-FU regimen was different from ours. We felt very strongly that further confirmation was required. A national intergroup study was therefore undertaken involving the Eastern Cooperative Oncology Group, the NCCTG, and the Southwest Oncology Group. This trial, which essentially duplicates the methods of the study reported here, has enrolled over 1,200 patients, all of whom completed their year of therapy by October 1987. We hope that preliminary results may be available in 1 to 2 years.

ACKNOWLEDGMENT

Additional participating institutions include The St. Cloud Clinic of Internal Medicine, Ltd, St. Cloud, MN, and Quain and Ramstad Clinic, Bismarck, ND.

REFERENCES

1. Moertel CG: Trials, errors and glimmers of success in the surgical adjuvant treatment of colorectal cancer. Levin B (ed): Gastrointestinal Cancer. Austin, TX, University of Texas Press, 1988, pp 3-17

2. Buyse M, Zelenuich-Jacquotte A, Chalmers TC: Adjuvant therapy of colorectal cancer. Why we still don't know. JAMA 259:3571-3578, 1988

3. Wolmark N, Fisher B, Rockette H, et al: Postoperative adjuvant chemotherapy or BCG for colon cancer. J Natl Cancer Inst 1:30-36, 1988

4. Moertel CG: Surgical adjuvant treatment of large bowel cancer. J Clin Oncol 6:934-939, 1988

5. Mansour E, Ryan L, Lerner H, et al: Lack of effectiveness of 5-FU + methyl CCNU as compared to 5-FU for adjuvant therapy in colon cancer. Proc Am Soc Clin Oncol 8:115, 1989

6. Panettiere FJ, Goodman PJ, Costanzi JJ, et al: Adjuvant therapy in large bowel adenocarcinoma. J Clin Oncol 6:947-954, 1988

7. Gastrointestinal Tumor Study Group: Adjuvant therapy of colon cancer. N Engl J Med 310:737-743, 1984

8. Souter RG, Gill PG, Morris PJ: A trial of non-specific immunotherapy Using systemic C. parvum treated patients with dukes B and C colorectal cancer. Br J Cancer 45:506-512, 1982

9. Taylor J, Machin D, Mullee M, et al: A randomized controlled study of adjuvant portal vein cytotoxic perfusion in colorectal cancer. Br J Surg 72:359-363, 1984

10. Verhaegen H, DeCree J, DeCock W, et al: Levamisole therapy in patients with colorectal cancer, in Terry WD, Rosenberg SA (eds): Immunotherapy of Human Cancer. New York, NY, Elsevier, 1982, pp 225-229

11. Chirigos MA, Amery WK: Combined levamisole therapy: An overview of its protective effects, in Immunotherapy of Human Cancer. New York, NY, Raven, 1978, pp 181-195

12. Renoux G: The general immunopharmacology of levamisole. Drugs 20:89-99, 1980

13. Grage TB, Moss SE: Adjuvant chemotherapy in

cancer of the colon and rectum: Demonstration of effectiveness of prolonged 5-FU chemotherapy in a prospectively controlled randomized trial. Surg Clin North Am 61:1321-1329, 1981

14. Laurie J, Moertel C, Fleming T, et al: Surgical adjuvant therapy of poor prognosis colorectal cancer with levamisole alone or combined levamisole and 5-fluorouracil (5-FU). Proc Am Soc Clin Oncol 5:316, 1986 (abstr)

15. SAS Program Institute Inc, SUGI: Supplemental Library Users' Guide. (ed 5). Cary, NC, SAS Institute, 1986, pp 662

16. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958

17. Mantel H, Haenszel W: Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 22:719-748, 1959

18. Cox DR: Regression models and life tables. J Roy Statis Soc B 34:187-202, 1972

19. Klefstrom P, Holsti P, Groha P, et al: Levamisole in the treatment of stage II breast cancer. Cancer 55:2753-2757, 1985

20. Treurniet-Donker AD, Meiscke de Jongh ML, van Puttens WLJ: Levamisole as adjuvant immunotherapy in breast cancer. Cancer 59:1590-1593, 1987

21. Danish Breast Cancer Cooperative Group: Increased breast cancer recurrence rate after adjuvant therapy with levamisole. Lancet 2:824-827, 1980

22. Amery WK, Cosemans J, Goossen HC, et al: Four year results from double-blind study of adjuvant levamisole treatment in resectable lung cancer, in Terry WE, Rosenberg SA (eds): Immunotherapy of Human Cancer. New York, NY, Elsevier, 1982, pp 123-133

23. Van Houtte P, Bondue H, Rocmans P, et al: Adjuvant immunotherapy by levamisole in resectable lung cancer. Eur J Cancer 16:1597-1601, 1980

24. Anthony HM, Mearns AJ, Masono MK, et al: Levamisole and surgery in bronchial carcinoma patients. Thorax 34:4-12, 1979

25. Herskovic A, Bauer M, Seydel HG, et al: Postoperative thoracic irradiation with or without levamisole in non-small cell lung cancer. Int J Radiat Oncol Biol Phys 14:37-42, 1988

26. Quirt I, Shelley W, Bodurtha A, et al: Adjuvant levamisole improves survival and disease free survival in patients with poor prognosis malignant melanoma. Proc Am Soc Clin Oncol 5:130, 1986 (abstr)

27. Spittler LE, Sagebeil R, Allen R, et al: Levamisole in the treatment of melanoma, in Terry WD, Rosenberg SA (eds): Immunotherapy of Human Cancer. New York, Elsevier, 1982, pp 289-297

28. House AK, Maley MAL: Clinical and in vivo response following surgery or surgery plus adjuvant chemotherapy or immunotherapy for colorectal carcinoma in a rat model. J Royal Soc Med 76:833-840, 1983

29. Wesse JL, Gilbertson EM, Syrjala SE: Prevention of rat colon cancer metastases by perioperative immunostimulation. Surgery 96:420-426, 1984

30. Martin MS, Justrabo E, Martin F, et al: Ineffectiveness of levamisole as adjuvant to surgery with two lines of transplanted rat colonic carcinoma. Br J Cancer 44:464-466, 1981

31. Chlebowski RT, Nystrom S, Reynolds R, et al: Long-term survival following levamisole or placebo adjuvant treatment of colorectal cancer. Oncology 45:141-143, 1988

32. Windle R, Bell PRF, Shaw D: Five year results of a randomized trial of adjuvant 5-fluorouracil and levamisole in colorectal cancer. Br J Surg 74:569-572, 1987

33. Harris BG: Levamisole: Mode of action. Biochem Soc Trans 15:61, 1987

34. Goldin A, Chirigos MA, Macdonald JS, et al: Biologic response modifiers and adjuvant chemotherapy. Recent Results Cancer Res 80:351-356, 1982

35. Borden EC, Davis TE, Crowley JJ, et al: Interim analysis of a trial of levamisole and 5-fluorouracil in metastatic colorectal carcinoma, in Terry WD, Rosenberg SA (eds): Immunotherapy of Human Cancer. New York, NY, Elsevier, 1982, pp 231-235

36. Buroker TR, Moertel CG, Fleming TR, et al: A controlled evaluation of recent approaches to biochemical modulation or enhancement of 5-fluorouracil therapy in colorectal carcinoma. J Clin Oncol 3:1624-1631, 1985

EXHIBIT F

LEVAMISOLE AND FLUOROURACIL FOR ADJUVANT THERAPY OF RESECTED COLON CARCINOMA

CHARLES G. MOERTEL, M.D., THOMAS R. FLEMING, PH.D., JOHN S. MACDONALD, M.D., DANIEL G. HALLER, M.D., JOHN A. LAURIE, M.D., PHYLLIS J. GOODMAN, M.S., JAMES S. UNGERLEIDER, M.D., WILLIAM A. EMERSON, M.D., DOUGLAS C. TORMEY, M.D., JOHN H. GLICK, M.D., MICHAEL H. VEEDER, M.D., AND JAMES A. MAILLIARD, M.D.*

Abstract Twelve hundred ninety-six patients with resected colon cancer that either was locally invasive (Stage B_2) or had regional nodal involvement (Stage C) were randomly assigned to observation or to treatment for one year with levamisole combined with fluorouracil. Patients with Stage C disease could also be randomly assigned to treatment with levamisole alone. The median follow-up time at this writing is 3 years (range, 2 to 5½).

Among the patients with Stage C disease, therapy with levamisole plus fluorouracil reduced the risk of cancer recurrence by 41 percent ($P<0.0001$). The overall death rate was reduced by 33 percent ($P = 0.006$). Treatment with levamisole alone had no detectable effect. The results in the patients with Stage B_2 disease were equivocal and too preliminary to allow firm conclusions. Toxic effects of levamisole alone were infrequent, usually consisting of mild nausea with occasional dermatitis or leukopenia, and those of levamisole plus fluorouracil were essentially the same as those of fluorouracil alone — i.e., nausea, vomiting, stomatitis, diarrhea, dermatitis, and leukopenia. These reactions were usually not severe and did not greatly impede patients' compliance with their regimen.

We conclude that adjuvant therapy with levamisole and fluorouracil should be standard treatment for Stage C colon carcinoma. Since most patients in our study were treated by community oncologists, this approach should be readily adaptable to conventional medical practice. (N Engl J Med 1990; 322:352-8.)

THIS year, cancer of the colon will afflict over 100,000 persons in the United States.[1] As a cause of death due to cancer, it is second only to lung cancer. There is no established means of preventing colon cancer, and there is no reliable and cost-effective means of screening to ensure early diagnosis. In the main, symptomatic patients must be treated as they present themselves, and in half of them cure has unfortunately not been possible. However, in about 80 percent of patients the diagnosis is made at a stage when all apparent diseased tissue can be surgically removed. In such patients, incurability is likely to be due to residual cancer existing in an occult and probably microscopic stage. Studies of animal models of the disease indicate that at such a stage, effective chemotherapy or immunotherapy is most likely to result in cure. Numerous randomized, controlled trials of adjuvant therapy for resected colon cancer have used various forms of immunotherapy and chemotherapy, often fluorouracil either alone or in combination with other agents. Although these trials have involved several thousand patients, there has been no convincing and reproducible evidence of significant benefit.[2] The consensus has seemed to be that the best standard treatment was surgery alone, and thus that the use of untreated controls was ethically justified in any surgical adjuvant trial.

In 1989 a report from the North Central Cancer Treatment Group (NCCTG) suggested a possible benefit from the use of levamisole, either alone or in combination with fluorouracil, as adjuvant therapy for colorectal cancer in Dukes' Stage B_2 (invasion of serosa or pericolonic fat) or Stage C (metastasis to regional lymph nodes).[3]

Levamisole has had extensive, worldwide use as an anthelmintic drug in both humans and domestic animals.[4] It attracted interest as an agent for cancer therapy because of its presumed immunomodulatory activity.[4,5] Its use in combination with fluorouracil in the NCCTG study was based on the hope that it would add to the marginal activity of fluorouracil.[3] The results of that trial, which had a median follow-up time of seven years, showed that adjuvant therapy with the combination of levamisole and fluorouracil significantly reduced the cancer-recurrence rate as compared with the rate when no adjuvant therapy was given, whereas the use of levamisole alone produced a borderline advantage. Analysis of overall survival showed a suggestive but not definite advantage of the combination therapy. Subset analysis, however, did show that the treatment conferred a significant advantage for survival on patients with Stage C disease. These results were sufficiently promising to justify a larger and more definitive study. The methods of this confirmatory trial were nearly identical to those of the NCCTG study, except that, to avoid possible confusion due to subset analysis, sepa-

From the Mayo Clinic, Rochester, Minn. (C.G.M.), the Fred Hutchinson Cancer Research Center, Seattle (T.R.F., P.J.G.), Temple University School of Medicine, Philadelphia (J.S.M.), the University of Pennsylvania Cancer Center, Philadelphia (D.G.H., J.H.G.), Grand Forks Clinic, Grand Forks, N.D. (J.A.L.), Dayton Clinical Oncology Program, Dayton (J.S.U.), St. Louis Community Clinical Oncology Program, St. Louis (W.A.E.), Wisconsin Clinical Cancer Center, Madison (D.C.T.), Illinois Oncology Research Association, Peoria (M.H.V.), and Creighton University, Omaha (J.A.M.). Address reprint requests to Dr. Moertel at the Mayo Clinic, Rochester, MN 55905

Supported in part by grants (CA-25224, CA-31224, and CA-37404 [to the North Central Cancer Treatment Group and Mayo Clinic], CA-21115, CA-37403, and CA-39088 [to the Eastern Cooperative Oncology Group], CA-32102, CA-37429, and CA-39091 [to the Southwest Oncology Group], and CA-38926 [to the Fred Hutchinson Cancer Research Center]) from the U.S. Public Health Service, and by grants to individual participating institutions from the National Cancer Institute.

*For the Eastern Cooperative Oncology Group, the North Central Cancer Treatment Group, the Southwest Oncology Group, and the Mayo Clinic.

rate studies were conducted for patients in Stage B_2 and those in Stage C.

Methods

This was a national intergroup trial that was sponsored by the National Cancer Institute and involved the Eastern Cooperative Oncology Group, the NCCTG, the Southwest Oncology Group, and the Mayo Clinic. Enrollment of patients was begun in March 1984, when a preliminary analysis of the NCCTG study[1] indicated the likelihood of a treatment advantage for levamisole plus fluorouracil and for levamisole alone, with regard to time to recurrence. Enrollment was completed in October 1987.

Patient Selection

All patients were required to have undergone a potentially curative en bloc resection of an adenocarcinoma of the colon without gross or microscopic evidence of residual disease. Patients with rectal carcinoma were ineligible. The resected specimen in eligible patients showed one of two indicators of poor prognosis — invasion extending at least to the serosa or pericolonic fat (Stage B_2) or metastasis to regional lymph nodes (Stage C). It was further required that the patient be able to swallow oral medication and have a leukocyte count of at least 4000 per microliter and a platelet count of at least 130,000 per microliter. Patients were ineligible if they had had any other cancer within five years except for superficial skin carcinoma or in situ carcinoma of the cervix. Eligibility was determined by careful review of study forms, operative reports, and pathology reports. Entry into the study was allowed no earlier than one week and no later than five weeks after surgery.

Stratification and Randomization Procedures

Written informed consent was obtained from the patients before they were enrolled in the study. Patients with B_2 lesions were stratified according to the extent of invasion (only into or through the serosa vs. into adjacent organs) and the interval since surgery (7 to 20 days vs. 21 to 35 days). They were then randomly assigned to either observation or therapy with levamisole plus fluorouracil. A dynamic randomization method was employed.[6]

Patients with Stage C lesions were stratified according to the invasion by the primary lesion and the interval since surgery and also according to the number of lymph nodes involved (≤ 4 vs. >4). They were then randomly assigned to observation, therapy with levamisole alone, or therapy with levamisole plus fluorouracil.

Protocol Management

Within 72 hours before randomization a medical history was taken and the following were performed: a physical examination, hematologic testing (hemoglobin measurement, leukocyte count, platelet count, and differential count), a blood-chemistry panel (including at least measurement of bilirubin, alkaline phosphatase, aminotransferases, and creatinine), and chest radiography if it had not been performed preoperatively.

Patients assigned to the control arm were observed after surgery, with no planned treatment. During the first year they were evaluated every 12 weeks, during the second year every four months, and thereafter every six months, for a total of five years. These evaluations consisted of an interim history-taking and physical examination, hematologic testing, a blood-chemistry panel, and chest radiography. In addition, either a proctoscopic examination and radiography of the colon (barium enema) or a colonoscopic examination was performed at 24 weeks, 48 weeks, and annually thereafter. Follow-up was continued beyond five years but without formal protocol requirements.

Patients assigned to levamisole alone had periodic evaluations at the same time as those who received no adjuvant therapy. In addition, they received levamisole by mouth (50 mg every eight hours) for a period of three days; this was repeated every two weeks for one year. Hematologic testing and blood-chemistry panels were repeated every four weeks. If persistent dermatitis or leukopenia developed, levamisole was discontinued.

Patients assigned to levamisole plus fluorouracil were evaluated at the same time as those who received no adjuvant therapy, and were given levamisole in the same dose and on the same schedule as patients assigned to levamisole alone. In addition, no earlier than 21 days after surgery, they received fluorouracil by rapid intravenous injection (450 mg per square meter of body-surface area) daily for five consecutive days. Twenty-eight days after the start of this course, weekly treatment with fluorouracil was begun with an intravenous dose (450 mg per square meter) and continued for 48 weeks. Leukocyte counts were determined before each weekly dose. If stomatitis, diarrhea, or leukopenia developed, weekly fluorouracil treatment was deferred until the side effects subsided. If these side effects were moderate to severe, the dose of fluorouracil was reduced by 20 percent.

Statistical Analysis

Survival was the primary end point of this study; the time to recurrence was also determined. A minimum of 150 eligible patients per treatment arm was planned for the trial involving Stage B_2 disease and 300 patients per arm for Stage C. This ensured that the Stage B_2 trial would have a power of 0.90 to detect a ratio of the control-group hazard to the combination-therapy–group hazard of 2.0. This assumed a pairwise comparison of treatments by a one-sided log-rank test in which a value of 0.05 indicated statistical significance. The Stage C study would have a power of 0.90 to detect a hazard ratio of 1.35.

Statistical analyses were carried out according to the procedures of the Statistical Analysis System.[7] The survival curves were generated by the Kaplan–Meier method.[8] The log-rank statistic[9] was used to compare the distributions of survival times. The Cox proportional-hazards model[10] was used to determine the ratios of relapse and survival rates and to perform all multivariate analyses. Backward regression was used to find the significant prognostic factors; variables were progressively eliminated on the basis of the maximal partial-likelihood estimate (MLE) statistics. To adjust for covariates when evaluating treatments, we kept the variable of treatment in the model and used the backward regression for other covariates, keeping those whose MLE statistics satisfied the criterion of a P value less than 0.01. All P values reported for this study are two-sided.

Results were carefully monitored with periodic formal analyses of survival, recurrence, and other secondary outcomes. Consideration was given at these times to possible early reporting of results. We used the four-stage group sequential boundary of O'Brien and Fleming[11] with an error rate of 0.05, since we thought it clear that early termination should occur only if the results were extreme. Analyses were planned to occur after approximately 125, 250, 375, and 500 deaths were observed among patients with Stage C disease. Any decision about early termination and early reporting was planned to be global in nature, taking into account not only overall survival but also the characteristics of the patients treated, overall recurrence, toxicity, and relevant results reported by other investigators. At the second planned interim analysis in September 1989, the results for survival met the protocol criteria for early reporting. To be specific, because this interim analysis was performed after 301 deaths were observed among patients in Stage C, the O'Brien–Fleming criterion required the two-sided P value to be less than 0.0098 — i.e., $2\,\Phi\,[-4.006\,(125/301)^{1/2}]$, where Φ denotes the cumulative distribution function for the standard normal distribution (see Table 1 of Emerson and Fleming).[12] This criterion, however, was met only in the Stage C part of the study, which is reported in detail. Although the results of the Stage B part of the study will be described in brief, we regard these as inconclusive.

Results

A total of 1296 patients were entered in this trial. Of 325 patients entered in the Stage B_2 study, 7 (2.2 percent) were considered ineligible (2 assigned to observation and 5 to levamisole plus fluorouracil). Of 971 patients entered in the Stage C study, 42 (4.3 percent) were ineligible (12 assigned to observation,

18 to levamisole alone, and 12 to levamisole plus fluorouracil). Ineligibility was most frequently due to the presence of a stage of disease more advanced than that allowed by the protocol. Because ineligibility was not biased by treatment assignment, these patients were excluded from the analysis. Eight patients in the Stage B_2 study and 14 in the Stage C study refused to accept their treatment assignment. Of those who refused in the Stage B study, 5 (63 percent) were assigned to the treatment arm; 13 of 14 (93 percent) of those who refused in the Stage C study were assigned to the observation arm. Because this withdrawal from study could be and undoubtedly was biased by treatment assignment, these patients were included in all analyses. At present, follow-up findings in 98 percent of the patients have been reported in the timely fashion specified by the protocol.

The median follow-up time for this study is now 3 years (range, 2 to 5½). On the basis of the original projections of our protocol, we estimate that among patients with Stage B_2 disease, 80 percent of the anticipated recurrences in the control arm have been recorded but only 27 percent of the deaths expected to occur during the first five years. Therefore, from the standpoint of survival these are rather early results. On the other hand, results in the Stage C study show that 82 percent of the anticipated recurrences have occurred, as well as 60 percent of the anticipated deaths.

Table 1. Clinical and Pathological Characteristics in the Stage C Colon-Cancer Study.

	Observation (N = 315)	Levamisole (N = 310)	LEV + 5-FU (N = 304)
Age — median (range)	60 (18–84)	61 (26–83)	61 (25–80)
		percent of patients	
Sex — male	53	57	46
Days since surgery			
7–20	29	26	25
21–35	71	74	75
Location of primary tumor			
Cecum and right colon	31	35	34
Flexures and transverse colon	14	19	17
Left colon	6	5	4
Sigmoid and rectosigmoid	47	38	43
Multiple primaries	3	3	2
Depth of invasion			
Submucosa	3	1	3
Muscular layer	12	12	10
Serosa	85	87	86
Adjacent organ involvement			
Adhesions	15	16	13
Invasion	8	7	3
Obstruction	20	20	18
Perforation	3	4	3
Regional peritoneal implants	5	6	7
No. of nodes involved			
1–4	72	71	74
>4	28	29	26
Histologic differentiation			
Well	9	12	10
Moderately well	73	71	71
Poor	17	14	18
Unknown	2	3	2

Stage B_2 Study

The characteristics of the 318 patients in this study are well balanced between the protocol arms. At the time of this writing, 54 patients have had recurrences (32 on the observation arm and 22 on the levamisole–fluorouracil arm). At 3½ years, 84 percent of the patients who received levamisole plus fluorouracil and 77 percent of the patients who underwent observation are free of recurrence according to Kaplan–Meier estimates. There has been a disproportionate number of deaths due to causes unassociated with recurrence on the levamisole–fluorouracil arm (six, as compared with only one on the observation arm). The preliminary data on survival indicate that 29 patients have died — 18 among those receiving levamisole plus fluorouracil. The survival estimates at ½ years are 91 percent in the control group and 85 percent in the levamisole–fluorouracil group. However, 22 patients in the control group and 10 in the levamisole–fluorouracil group have had recurrence of cancer but are still living. At least an additional two years of observation will be required before definite conclusions can be drawn.

Stage C Study

The characteristics of the 929 eligible patients with Stage C disease are shown in Table 1. In the main, they were well balanced among the study arms. More men received levamisole alone, and fewer received levamisole plus fluorouracil. There were more patients on the observation arm who had tumors of the sigmoid and rectosigmoid and lesions invading adjacent organs.

At present, 402 patients have had recurrences. Of these, 155 are in the observation group, 144 in the levamisole group, and 103 in the levamisole–fluorouracil group. The estimated overall reduction in the recurrence rate (Cox proportional-hazard model) with levamisole–fluorouracil therapy is 41 percent (95 percent confidence interval, 23 to 54 percent). At 3½ years, 63 percent of the patients receiving levamisole plus fluorouracil and 47 percent of the control patients are free of recurrence according to Kaplan–Meier estimates. Recurrence-free intervals are plotted in Figure 1 and are carried out to 52 months, at which point fewer than 10 percent of the patients can be followed. Therapy with levamisole plus fluorouracil produced an unequivocal advantage over observation ($P<0.0001$). On the other hand, therapy with levamisole alone produced no detectable effect.

Figure 2 shows the effect of therapy with levamisole plus fluorouracil on specific sites of initial recurrence. The rates of recurrence were reduced for all sites. This was most striking (>50 percent) for sites outside the abdominal cavity — i.e., the lungs, retroperitoneal nodes, peripheral nodes, and abdominal wall. It is possible that the more distant sites had a smaller, less well established tumor burden at the time that adjuvant therapy began.

Table 2 shows the relation of the patients' patho-

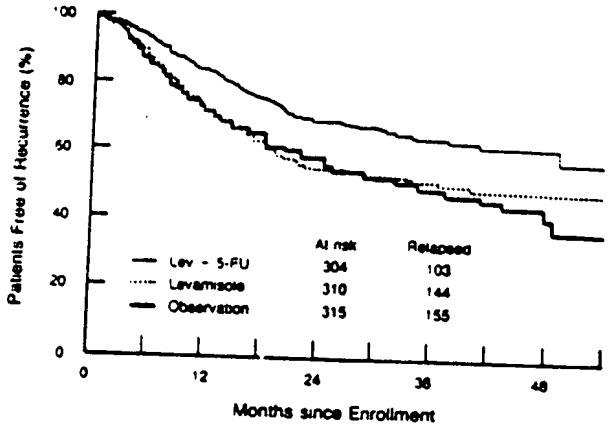


Figure 1. Recurrence-free Interval, According to Study Arm. Lev + 5-FU denotes combination therapy with levamisole and fluorouracil.

logical characteristics to recurrence. By a backward-regression selection procedure, the depth of invasion, the number of metastatic lymph nodes, and histologic differentiation were all found to be independent determinants of recurrence ($P<0.01$). After adjustment for imbalances among prognostic variables, therapy with levamisole plus fluorouracil was again found to have a significant advantage over observation in terms of preventing recurrence ($P = 0.0002$). Levamisole alone had no significant advantage ($P = 0.64$).

In these analyses, data on patients who died without recurrences were censored. Comparable results were observed when death without recurrence was considered an event — i.e., recurrence-free survival.

At present, 301 patients have died: 114 on the observation arm, 109 who received levamisole alone, and 78 who received levamisole plus fluorouracil. The estimated reduction in the death rate by treatment with levamisole plus fluorouracil as compared with observation is 33 percent (95 percent confidence interval, 10 to 50 percent). Eighteen patients died without evidence of recurrence: five on the observation arm, seven who received levamisole alone, and six who received levamisole plus fluorouracil. As would be anticipated in patients with this age distribution, these deaths were largely cardiovascular (11 patients). There are 119 patients who have documented recurrence but are still alive: 46 of these on the observation arm, 42 who received levamisole alone, and 31 who received levamisole plus fluorouracil. These figures make it likely that the survival advantage of treatment with levamisole plus fluorouracil will be sustained. Survival among all eligible patients in the study is plotted in Figure 3. The 3½-year survival estimates were 55 percent for the observation arm and 71 percent for the levamisole–fluorouracil arm. Whereas survival with levamisole therapy alone overlapped that with observation, survival with levamisole plus fluorouracil showed that this treatment had a decided advantage ($P = 0.0064$). This difference exceeded our protocol definition of extreme results that would justify early reporting — i.e., a two-sided P value of less than 0.0098.

The relations between the characteristics of the patients or their cancers and survival (Table 2) were similar to the relations between these characteristics and recurrence. After variables were selected by backward regression, the following characteristics were found to have independent prognostic significance ($P<0.01$): the location of the primary tumor, the depth of invasion, obstruction, the number of metastatic nodes, and histologic differentiation. When the proportional-hazard model was used to correct for the influence of prognostic variables, levamisole plus fluorouracil was again found to have a significant survival advantage over observation ($P = 0.0052$). Levamisole alone showed no effect ($P = 0.92$).

In exploratory subset analyses, levamisole–fluorouracil treatment appeared to have the greatest advantage among male patients (in both survival and recurrence), older patients (recurrence), patients with tumors that were well differentiated to moderately well differentiated (survival and recurrence), patients in whom more than four nodes were involved (survival), and patients treated 21 to 35 days after surgery (recurrence). These results show two striking contradictions to those of subset analyses reported in the NCCTG study, in which levamisole plus fluorouracil was found to be most effective in reducing the risk of recurrence among female patients and younger patients. This underscores the importance of the statement by the authors of that study, that "subset analyses must be interpreted with great caution."[1]

Toxicity

Toxic reactions are presented according to treatment arm in Table 3. For this analysis we have grouped data from the studies of Stages B, and C disease. The reactions to levamisole alone were typically mild. One patient, however, had a life-threatening exfoliative dermatitis. A characteristic reaction to levamisole that probably occurred more often than it was recorded was an unusual taste, usually described as metallic and occasionally associated with an altered sense of smell. Uncommon reactions, seemingly co-

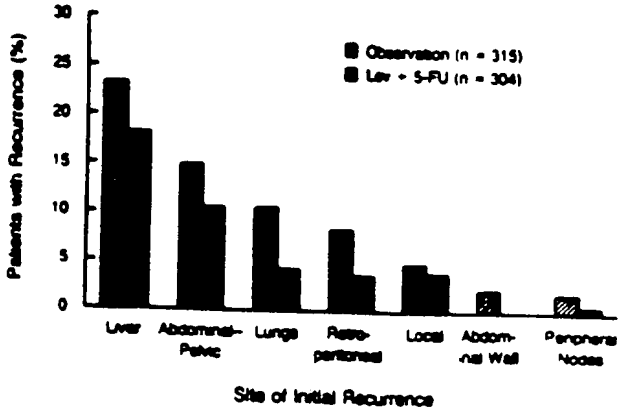


Figure 2. Site of Initial Recurrence, According to Study Arm

incident with the days of treatment, were arthralgia and myalgia. Mood-altering effects have been attributed to levamisole,[4] and a small proportion of patients experienced anxiety, irritability, depression, somnolence, or insomnia. It was difficult to determine whether these symptoms were in fact treatment-related. Hematologic depression was infrequent and fully reversible. One patient had a minor rise in the bilirubin level at eight months without an accompanying change in liver enzyme levels.

The predominant toxic reactions to levamisole plus fluorouracil were those that might have been anticipated with fluorouracil alone — i.e., nausea, vomiting, diarrhea, stomatitis, dermatitis, and leukopenia. These reactions were rarely severe. Some degree of alopecia was experienced by 22 percent of the patients, but it was judged to be severe in only 2 percent. A variety of neurologic symptoms were experienced by 83 patients (18 percent). These ranged from vague lightheadedness and emotional changes to disabling cerebellar ataxia, and they usually abated when therapy was discontinued.

Eleven patients had evidence of hepatic toxicity (elevated levels of bilirubin, nine patients; alkaline phosphatase, seven patients; and aminotransferases, three patients). In 10 patients these changes occurred during maintenance therapy, reaching a maximum at a median time of 7½ months. In all instances there was improvement after therapy was discontinued, usually to a completely normal state (nine patients).

Leukopenia was the toxic reaction that usually led to dose limitation. It was seldom severe, but the single drug-related death observed in this study was due to profound leukopenia and sepsis.

Second Primary Cancers

Second primary cancers have been documented in 35 of the 1247 patients in this study: 15 of 474 (3.2 percent) in the observation group, 5 of 310 (1.6 percent) in the levamisole group, and 15 of 463 (3.2 percent) in the levamisole-plus-fluorouracil group. As might be expected, the large bowel was the most frequent site (nine cases). A single case of leukemia (acute lymphoblastic) was observed in the group that received levamisole alone, and this first became evident after only three months of therapy. Leukemogenesis has not been a recognized problem in previous trials with levamisole, and among almost 900 patients treated with levamisole or levamisole plus fluorouracil in the present study and the NCCTG study, this is the only documented case.

Table 2. Influence of Prognostic Variables on Recurrence and Survival (Stage C Study).

Variable	No. of Patients	Percent Recurrence-free at 3½ Yr	P Value	Percent Surviving by 3½ Yr	P Value
Sex					
Male	484	55	0.104	59	0.999
Female	445	51		61	
Age					
<61 yr	470	54	0.883	62	0.188
≥61 yr	459	52		58	
Days since surgery					
7–20	247	49	0.082	59	0.098
21–35	682	54		61	
Location of primary tumor*					
Cecum and right colon	308	52	0.111	53	<0.001
Flexures and transverse colon	153	50		51	
Left colon	49	60		64	
Sigmoid and rectosigmoid	397	55		68	
Multiple sites	24	42		67	
Depth of invasion*†					
Submucosa or muscular layer	127	76	<0.001	84	<0.001
Serosa	802	49		56	
Adhesion to adjacent organs					
Yes	58	46	0.068	45	0.020
No	871	54		63	
Invasion of adjacent organs					
Yes	58	43	0.006	29	0.006
No	871	54		61	
Obstruction*					
Yes	180	46	0.019	47	<0.001
No	749	55		63	
Perforation					
Yes	27	36	0.078	54	0.716
No	902	53		60	
Regional peritoneal implants					
Yes	57	38	0.006	42	0.001
No	872	54		61	
No. of nodes involved*†					
1–4	674	61	<0.001	70	<0.001
>4	255	33		34	
Histologic differentiation*†					
Well	93	56	<0.001	57	<0.001
Moderately well	663	55		64	
Poor	150	40		42	

*Independent prognostic variable for survival, P<0.01. †Independent prognostic variable for recurrence, P<0.01.

Treatment Compliance

Compliance with levamisole therapy was monitored both through questioning of the patients by physicians and nurses and through a drug diary kept by the patient. Of the 310 patients assigned to levamisole alone, 286 (92 percent) continued treatment for at least 90 percent of the scheduled year or until death or disease progression. Among the 24 patients in whom therapy was abbreviated, the most common reason was drug toxicity (11 patients) and the most frequent specific side effect was arthralgia (6 patients).

In view of the greater toxicity and practical problems of the levamisole–fluorouracil regimen, it is not surprising that a larger proportion of patients prematurely discontinued treatment (136 of 457, 30 percent), after a median of five months. In 56 of these patients,

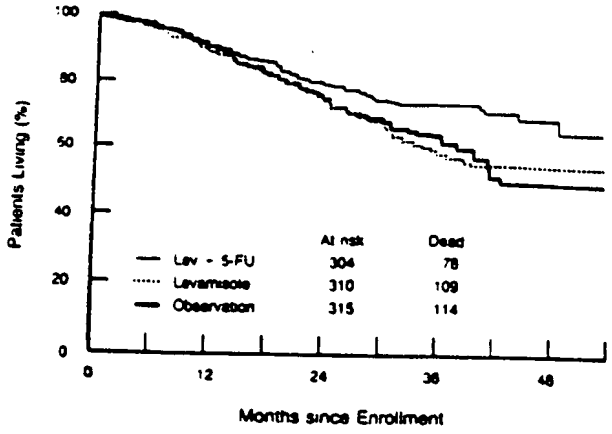


Figure 3. Survival, According to Study Arm.

toxicity was the principal reason, and the usual specific side effect was nausea.

Discussion

The results of this study indicate that therapy with levamisole plus fluorouracil reduces recurrence rates in patients with surgically treated Stage C colon carcinoma. This reduction in recurrence rates should lead to a reduction in deaths due to cancer. There is also evidence that treatment with levamisole plus fluorouracil significantly reduces the overall death rate, at least during the first 3½ years after surgery. These results confirm the significant overall reduction in recurrence rates and the reduction in death rates among patients with Stage C disease (significant only in subset analysis) observed in the NCCTG study.[3]

The improvement produced by levamisole plus fluorouracil was not only statistically significant but also clinically meaningful. The reduction in recurrence and death rates by approximately one third is substantial and justifies the inconvenience of therapy as well as the usually tolerable toxicity.

Approximately 21,000 patients will have surgical treatment for Stage C colon cancer in this country over the next year (Surveillance, Epidemiology and End Results Program, National Cancer Institute: unpublished data). Not all these patients will be suitable for or will desire adjuvant therapy, but there are very few contraindications to this treatment and it seems reasonable for physicians to offer this option. This study was not confined to major cancer centers or university hospitals. The majority of patients were fully treated in community practice. It is therefore reasonable to assume that therapy with levamisole plus fluorouracil could easily be incorporated into standard medical practice, with results comparable to those in this study.

Our results are still too early to allow assessment of the effectiveness of levamisole plus fluorouracil in patients with Stage B_2 cancer. There is a suggestion of reduced recurrence rates, but this has not been accompanied by any improvement in survival. Although advantages in both recurrence and survival were shown in patients with this stage in the NCCTG study, the numbers of patients were small and the differences were not significant. Also, neither the current study nor the NCCTG study offers evidence of the effectiveness of levamisole plus fluorouracil for rectal carcinoma, in which local recurrence is a major problem and emerging evidence indicates that multimodality approaches incorporating radiation therapy may be more rational and more effective.[13,14]

It is difficult to explain why this empirically conceived drug combination is effective. In advanced colorectal cancer, a preliminary report of a small study showed a suggestive but not significant advantage among patients given levamisole plus fluorouracil as compared with those treated with fluorouracil alone.[15] A larger and more mature NCCTG trial of a similar regimen, however, showed no survival advantage.[16] It is possible that the results of the present study could have been obtained with fluorouracil alone, but this seems unlikely in view of past experience. Although tested as a single agent in several trials of therapy adjuvant to colon surgery, fluorouracil has never been

Table 3. Toxic Effects, According to Treatment Arm.

Effect	Levamisole (N = 310)	Levamisole plus Fluorouracil	
		Induction (N = 657)	Maintenance (N = 441)*
		percent of patients	
Gastrointestinal			
Nausea	24	37	56
Severe	1	2	5
Vomiting	7	8	17
Severe	1	2	2
Diarrhea	13	25	47
Severe	1	3	7
Mucocutaneous			
Stomatitis	3	27	28
Severe	0	5	3
Dermatitis	9	8	22
Severe	2	1	1
Alopecia	3	4	22
Severe	0	1	2
Conjunctivitis	1	1	7
Hematologic†			
Leukopenia			
<4000–≥2000	8	38	38
<2000–≥1000	1	4	2
<1000	0	3	0
Thrombocytopenia			
<130,000–≥50,000	2	4	18
<50,000–≥25,000	1	1	3
<25,000	0	1	1
Other			
Fatigue or weakness	8	5	11
Taste change	7	2	7
Arthralgia or myalgia	7	2	4
Headache	3	1	3
Dizziness or vertigo	2	1	4
Ataxia	0	0	3
Anxiety or irritability	3	2	2
Impaired liver function	1	1	2

*Excludes patients who did not receive maintenance therapy
†Excludes patients in whom counts were not adequately documented

markedly effective. When all studies of fluorouracil were evaluated in a meta-analysis of 3499 patients, there was only a minor increase in five-year survival (3.4 percent), which barely reached statistical significance ($P = 0.04$).[2] In a small study using a different dosage regimen, patients treated with the combination of levamisole and fluorouracil were reported to have a significant survival advantage not only over untreated controls but also over patients treated with fluorouracil alone.[17] Although a few reports have claimed that the use of levamisole alone as an adjuvant to surgery had a positive effect on other cancers, these claims have been denied in confirmatory trials.[18] Our study and a recently reported trial of the European Organisation for Research on Treatment of Cancer[19] provide convincing evidence that levamisole alone is of little or no value in patients with colon cancer.

Although modulation of immunity has been the presumed mechanism for the antineoplastic activity of levamisole, this agent has a broad spectrum of pharmacologic activities, including the inhibition of fumarate reductase, potent inhibition of mammalian alkaline phosphatases, and inhibition of aerobic tumor glycolysis.[4,20] In animal models levamisole has been shown to improve survival when added to cyclophosphamide, semustine, and carmustine, and to do this in systems in which levamisole alone has no effect.[21] It is entirely possible that the clinical results we have obtained by adding levamisole to fluorouracil represent an example of biochemical modulation completely independent of immune effect. The possibility of biologic modulation is also raised by the finding that levamisole potentiates the activity of human interferon and interleukin-2.[22,23] The arbitrary nature of the dosage regimen for levamisole administration in this protocol probably dates back to the use of the drug as an anthelmintic. The purely empirical nature of the combination regimen we employed makes it likely that a more rational and effective combination regimen could be developed if the nature of the interaction between levamisole and fluorouracil were better known.[24]

We believe that this treatment should be offered to all patients with Stage C colon cancer who meet the eligibility criteria set down in our protocol. Since there appears to be no benefit of starting levamisole therapy soon after surgery, we recommend that therapy be deferred for a minimum of three weeks and begun then only if the patient is ambulatory, in a good state of nutrition, and free of postoperative complications. We have no evidence that starting therapy more than five weeks after surgery will produce any benefit.

We are indebted to Mrs. Deborah Papenfus and John VanDamme, B.S., B.A., whose expert and tireless efforts in data management and statistical assistance were invaluable to the conduct of this study.

References

1. Silverberg E, Lubera JA. Cancer statistics, 1989. CA 1989; 39:3-20.
2. Buyse M, Zelenuch-Jacquotte A, Chalmers TC. Adjuvant therapy of colorectal cancer: why we still don't know. JAMA 1988; 259:3571-8.
3. Laurie JA, Moertel CG, Fleming TR, et al. Surgical adjuvant therapy of large-bowel carcinoma: an evaluation of levamisole and the combination of levamisole and fluorouracil: the North Central Cancer Treatment Group and the Mayo Clinic. J Clin Oncol 1989; 7:1447-56.
4. Janssen PA. The levamisole story. Prog Drug Res 1976; 20:347-83.
5. Renoux G. The general immunopharmacology of levamisole. Drugs 1980; 19:89-99.
6. Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics 1975; 31:103-15.
7. SAS Institute Inc. SUGI supplemental library users' guide, version 5 edition. Cary, N.C.: SAS Institute, 1986:662.
8. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53:457-81.
9. Mantel H, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959; 22:719-48.
10. Cox DR. Regression models and life-tables. J R Stat Soc [B] 1972; 34:187-220.
11. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. Biometrics 1979; 35:549-56.
12. Emerson SS, Fleming TR. Symmetric group sequential test designs. Biometrics 1989; 45:905-23.
13. Gastrointestinal Tumor Study Group. Prolongation of the disease-free interval in surgically treated rectal carcinoma. N Engl J Med 1985; 312:1465-72.
14. Krook J, Moertel C, Wieand H, et al. Radiation vs sequential chemotherapy-radiation-chemotherapy: a study of the North Central Cancer Treatment Group, Duke University, and the Mayo Clinic. Proc Am Soc Clin Oncol 1986; 5:82. abstract.
15. Bordea EC, Davis TE, Crowley JJ, Wolberg WH, McKnight B, Chirigos MA. Interim analysis of a trial of levamisole and 5-fluorouracil in metastatic colorectal carcinoma. In: Terry WD, Rosenberg SA, eds. Immunotherapy of human cancer. New York: Excerpta Medica, 1982:231-5.
16. Buroker TR, Moertel CG, Fleming TR, et al. A controlled evaluation of recent approaches to biochemical modulation or enhancement of 5-fluorouracil therapy in colorectal carcinoma. J Clin Oncol 1985; 3:1624-31.
17. Windle R, Bell PR, Shaw D. Five year results of a randomized trial of adjuvant 5-fluorouracil and levamisole in colorectal cancer. Br J Surg 1987; 74:569-72.
18. Goldin A, Chirigos MA, Macdonald JS, Feder A, Mihich E. Biologic response modifiers and adjuvant chemotherapy: consideration of selected preclinical investigations in relation to clinical potential. Recent Results Cancer Res 1982; 80:351-6.
19. Arnaud J-P, Bayss M, Nordlinger B, et al. Adjuvant therapy of poor prognosis colon cancer with levamisole: results of an EORTC double-blind randomized clinical trial. Br J Surg 1989; 76:284-9.
20. Gumińska M, Kedryna T, Marchut E. Effect of levamisole on energy metabolism in Ehrlich ascites tumour cells in vitro. Biochem Pharmacol 1986; 35:4369-74.
21. Chirigos MA, Amery WK. Combined levamisole therapy: a overview of its promotive effects. In: Immunotherapy of human cancer: present status of trials in man. New York: Raven Press, 1978:181-95.
22. Munoz A, Garcia RA, Perez-Arnada A. Potentiation by levamisole, methisoprinol, and adenine or adenosine of the inhibitory activity of human interferon against encephalomyocarditis virus. Antimicrob Agents Chemother 1986; 30:192-5.
23. Redondo JM, Lopez-Guerrero JA, Presno M. Potentiation of interleukin-2 activity by levamisole and imidazole. Immunol Lett 1987; 14:111-6.
24. Green JL, Allegra CJ. Toxicity of levamisole and 5-fluorouracil in human colon carcinoma cells. J Natl Cancer Inst 1989; 81:1413-7.

served: hypertrophy and hyperplasia of the epithelium in the collecting duct in the outer medulla, and the accumulation of cytoplasmic droplets in the cells of the inner medulla and papilla. In addition, there may be tubular dilatation due to luminal obstruction by hyperplastic epithelial cells. It is important to note, however, that true renal cysts have not been observed, even after careful microdissection.[6] Finally, an interstitial reaction consisting of fibrosis and an inflammatory infiltrate of mononuclear cells often occurs after prolonged potassium depletion. The tubular changes (cytoplasmic droplets and hyperplastic lesions) are reversible after potassium repletion; however, the fibrosis and tubular dilatation persist.

The renal histologic picture of chronic potassium depletion in humans differs from that in rats in many respects. First, the principal site of changes in humans is the cortex, primarily the proximal tubular cells, in which prominent vacuolation of the cytoplasm occurs. Distal vacuolation of cortical tubular cells also occurs but is not as common. Second, although dilatation of the proximal tubules may be observed, there is no proliferation of the epithelium in the tubules. Third, there is no evidence of the cytoplasmic droplets observed in the medulla and papilla of the rat. Despite these differences between rats and humans, there is one important similarity — namely, the presence of interstitial fibrosis and chronic inflammation. As is the case with experimental potassium deficiency in rats, the renal tubular changes are reversible, but the fibrotic or inflammatory reaction is not. Finally, renal cystic lesions themselves have not been reported to occur in humans with chronic potassium deficiency of any cause.

In this issue of the *Journal*, Torres et al.[7] report the occurrence of reversible, predominantly medullary renal cysts in patients with chronic potassium depletion and primary or secondary aldosteronism. These findings suggest the intriguing possibility that chronic potassium depletion may engender cystic transformation of the medullary portion of the human kidney, perhaps due to epithelial hyperplasia of the collecting ducts, reminiscent of the hypokalemic lesion in rats.

The association between medullary cysts and potassium depletion is of great interest and should increase our understanding of the mechanisms of renal-cell growth and cyst formation. Cellular proliferation is a requirement for the generation of cysts. The association of potassium depletion with hypertrophy of proximal cells, and especially of the collecting-tubule cells of the outer medulla, suggests the possibility that cyst formation may be an unwelcome complication of increased cell growth. Decreases in the extracellular potassium concentration increase the growth rate of kidney cells in culture.[8] In addition, increased rates of ion transport in potassium deficiency may signal hypertrophy and cell division in some manner, explaining the frequent association between increased ion transport and growth in epithelial cells.

It seems unlikely that increased ion secretion, increased luminal flow, or both would lead to cystic dilatation, since the normal tubular lumen should easily tolerate marked increases in the rate of flow. Cysts have not been observed in patients with diabetes insipidus and markedly increased luminal flow rates. In addition, in potassium deficiency there is increased absorption of solutes (potassium bicarbonate) in the medullary collecting tubules rather than increased secretion. Finally, there is the intriguing possibility that ammonia could serve as an inducer of cyst formation. The production of ammonia is increased in potassium deficiency, and the largest accumulations are found in the renal medulla. It has been postulated that ammonia activates complement and causes interstitial nephritis.[9] In addition, ammonia increases the growth rate of renal cells in culture.[10]

The clinical entity of potassium deficiency has provided important clues to the role of cell pH in the regulation of processes such as the secretion of hydrogen by proximal tubules, the production of ammonia, and the absorption of citrate. It may also provide a model for understanding the relations between kidney function, growth, and cyst formation.

University of Texas
Southwestern Medical Center
Dallas, TX 75235-8856

ROBERT J. ALPERN, M.D.
ROBERT D. TOTO, M.D.

REFERENCES

1. Raymond KH, Lifschitz MD, McKinney TD. Prostaglandins and the urinary concentrating defect in potassium-depleted rabbits. Am J Physiol 1987; 253: F1113-F1119.
2. Soleimani M, McKinney TD. Potassium depletion increases Na/H exchange and Na:CO3:HCO3 cotransport in rat renal cortex. Clin Res 1989; 37:585A. abstract.
3. Wingo CS. Active proton secretion and potassium absorption in the rabbit outer medullary collecting duct: functional evidence for proton-potassium-activated adenosine triphosphatase. J Clin Invest 1989; 84:361-5.
4. Hays SR, Seldin DW, Kokko JP, Jacobson HR. Effect of K depletion on HCO_3 transport across rabbit collecting duct segments. Kidney Int 1986; 29:368. abstract.
5. Hollander W Jr, Blythe WB. Nephropathy of potassium depletion. In: Strauss MB, Welt LG, eds. Diseases of the kidney. 2nd ed. Boston: Little, Brown, 1971:933-72.
6. Oliver J, MacDowell M, Welt LG, et al. The renal lesions of electrolyte imbalance. I. The structural alterations in potassium-depleted rats. J Exp Med 1957; 106:563-73.
7. Torres VE, Young WF Jr, Offord KP, Hattery RR. Association of hypokalemia, aldosteronism, and renal cysts. N Engl J Med 1990; 322:345-51.
8. Walsh-Reitz MM, Toback FG. Kidney epithelial cell growth is stimulated by lowering extracellular potassium concentration. Am J Physiol 1983; 244:C429-C432.
9. Tolins JP, Hostetter MK, Hostetter TH. Hypokalemic nephropathy in the rat: role of ammonia in chronic tubular injury. J Clin Invest 1987; 79:1447-58.
10. Golchini K, Norman J, Bohman R, Kurtz I. Induction of hypertrophy in cultured proximal tubule cells by extracellular NH_4Cl. J Clin Invest 1989; 84:1767-79.

DOES ADJUVANT THERAPY WORK IN COLON CANCER?

COLORECTAL carcinoma is second only to lung cancer as a cause of death from cancer in the United States. Although the majority of patients found to have a large-bowel tumor successfully undergo a removal of all gross tumor, 40 to 50 percent are found at the time of surgery to have invasion of the tumor

through the serosa (Stage B_2) or spread to regional lymph nodes (Stage C). The prognosis after such "complete" resection is closely related to surgical stage. The prognosis for each stage appears to have improved somewhat in recent years, most likely because of more accurate surgical and pathological staging. The probability of five-year survival in patients operated on since 1960 is 70 to 75 percent among those with Stage B_2 tumors and ranges from 35 percent to 60 percent among those with Stage C disease, depending on the number of involved regional lymph nodes.[1] Because of their substantial risk of recurrence, patients with Stage B_2 or C disease have served as subjects in a series of trials of adjuvant therapy, evaluating the use of chemotherapy, radiation therapy, and immunotherapy.

As a rule, the only systemic agents that have been evaluated as adjuvant therapy are those previously found to be effective in the management of advanced disease. Since its introduction into clinical trials more than 25 years ago, fluorouracil remains the most commonly employed single agent in the treatment of patients with advanced colorectal cancer. Its use is associated with a likelihood of a partial response of 10 to 20 percent (i.e., more than a 50 percent reduction in the identifiable tumor mass). Attempts to enhance this modest response rate by combining fluorouracil with other marginally active drugs such as semustine, mitomycin, or both led to the transient impression that a regimen (MOF) of semustine (methyl-CCNU), vincristine (Oncovin), and fluorouracil was more effective than fluorouracil alone, thereby influencing the design of a generation of protocols for adjuvant therapy. Unfortunately, subsequent randomized trials including far larger numbers of patients demonstrated that neither the MOF regimen nor a regimen of mitomycin, semustine, and fluorouracil was superior to treatment with fluorouracil alone.[1]

The use as adjuvants of treatments found effective for advanced disease has been disappointing. The use of fluorouracil after resection of Stage B or C colorectal cancers has been compared with surgery alone in at least six controlled clinical trials. None of these studies revealed a definite improvement over the cure rate with surgery alone, and a recent meta-analysis of the available data also failed to provide unequivocal evidence of benefit.[2] The regimen of postoperative semustine and fluorouracil, with or without vincristine (i.e., MOF) and with or without a nonspecific immunostimulant (i.e., bacille Calmette–Guérin [BCG] or its methanol-extractable residue), has been compared with surgery with or without immunotherapy in at least five prospective randomized trials. Four of these trials failed to demonstrate a benefit for any adjuvant treatments; the fifth revealed a survival advantage of borderline significance in cohorts of patients given either MOF or BCG.[1] On the basis of these results, most investigators believed that postoperative treatment had not proved useful in patients with colon cancer and that more innovative therapeutic options were needed.

Levamisole represented such an option. An effective anthelmintic drug in humans and animals, levamisole has been shown to restore the function of macrophages and T lymphocytes after immunosuppression.[3] Because of this immunomodulatory action, levamisole was evaluated as an adjuvant agent to be given after the resection of a variety of malignant tumors;[4] at least one study suggested that this treatment benefited patients with large-bowel cancer.[5] On the basis of this preliminary information, investigators of the North Central Cancer Treatment Group (NCCTG) initiated a clinical trial in 1978 in which patients with Stage B_2 or Stage C colon cancer were randomly assigned within five weeks after their operative procedure to receive adjuvant fluorouracil and levamisole for one year, levamisole alone for one year, or observation only. After a median follow-up time of more than 7½ years, the patients with Stage C disease treated with fluorouracil and levamisole had fewer recurrences and a higher probability of survival than the control cohort. No benefit was observed in the patients with Stage B_2 tumors.[6] The NCCTG trial was relatively small, involving only 104 patients with Stage B_2 and 297 with Stage C tumors. Appropriately, the NCCTG researchers believed that the study required confirmation with far larger numbers of patients before any definite conclusions could be drawn. With the support of the National Cancer Institute, a national intergroup study was begun that included the NCCTG, the Eastern Cooperative Oncology Group, and the Southwest Oncology Group, and the earlier clinical trial was repeated in an essentially identical manner.

In this issue of the *Journal*, Moertel and coworkers present the initial results of this effort.[7] After a median follow-up time of three years, no benefit from the adjuvant treatment has yet been detected in the 318 patients with Stage B_2 lesions. In contrast, in the patients with Stage C disease, the use of adjuvant fluorouracil and levamisole was found to have reduced the recurrence rate by 41 percent and the probability of mortality by 33 percent when the cohort of 304 patients who were so treated was compared with 315 controls (observation only). The results observed in the 310 patients who received levamisole alone were the same as those in the control group. The adjuvant treatment did not require hospitalization and was generally well tolerated.

The outcome of this meticulously conducted study provides convincing evidence that adjuvant treatment is beneficial in patients with Stage C colon cancer. However, the elements essential to this benefit have not been clearly elucidated. What is the contribution (if any) of levamisole? Both the intergroup trial[7] and a recently concluded study performed by the European Organization for Research and Treatment of Cancer[8] have indicated that treatment with levamisole alone provides no adjuvant benefit in patients with Stage C colon cancer. In patients with metastatic colon cancer, a randomized trial failed to show that the addition of levamisole to fluorouracil improved the response rate or the duration of survival.[9] Laboratory studies

have shown no enhancement of cytotoxicity on human colon-cancer cells in culture when levamisole was added to fluorouracil.[10] Although these observations might be interpreted as reinforcing the concept of an immunomodulatory (rather than cytotoxic) effect of levamisole, it is possible (as the authors of the intergroup study acknowledge[7]) that the observed adjuvant effect of fluorouracil combined with levamisole may be attributable to fluorouracil alone, perhaps administered with better compliance among patients than when the previous trials of fluorouracil were conducted more than 20 years ago. The only investigation in which adjuvant fluorouracil therapy was compared with fluorouracil combined with levamisole in patients with colon cancer, although it suggested an advantage of the levamisole-containing combination, was compromised by the small number of its patients (only 41 of 141 had Stage C disease) and its markedly different method of administering both the fluorouracil and the levamisole.[11] In retrospect, the apparent conclusion of the intergroup study regarding levamisole could have been stated with far greater certainty if the protocol design had included the administration of fluorouracil alone as a treatment option.

The need for further assessment of levamisole has been made more compelling by the discovery that the efficacy of fluorouracil in patients with advanced colon cancer may also be enhanced by the addition of folinic acid (i.e., leucovorin or citrovorum factor). Fluorouracil is thought to affect cellular metabolism predominantly through competitive binding to thymidylate synthase, which results in the suppression of DNA synthesis through the depletion of thymidine. Laboratory studies suggest that the presence of reduced folates, such as folinic acid, enhances binding of fluorouracil to thymidylate synthase, thereby increasing the cytotoxic potential of the drug. In six of seven randomized trials conducted in patients with advanced colorectal cancer, the addition of folinic acid to fluorouracil led to marked improvement in the objective response rate as compared with fluorouracil alone.[1] As previously noted, the addition of levamisole to fluorouracil failed to induce such additional activity in similar patients with advanced disease.[9] Conceivably, a combination of fluorouracil and folinic acid might prove to be a far more effective adjuvant regimen than fluorouracil and levamisole, and comparisons of the two approaches (as well as an assessment of a combination of all three drugs) are in progress.

The intergroup trial[7] specifically excluded patients with rectal cancer. Carcinomas arising in the rectum (i.e., below the peritoneal reflection) are associated with a rate of local recurrence that is far higher than that observed for colon tumors. Previous studies had shown that postoperative chemotherapy and radiation therapy to the pelvis decrease the likelihood of local recurrence and prolong survival.[1] In the intergroup trial[7] it would therefore have been improper to offer patients with rectal cancer treatment with surgery alone. It is not known whether the addition of folinic acid or levamisole or both to chemotherapy and radiation therapy will prove beneficial in patients with rectal cancer; these issues are the focus of current studies of adjuvant therapy.

Available data do not yet support the use of adjuvant therapy in patients with Stage B_2 colon cancer or in patients with Stage C disease for whom more than five or six weeks have elapsed since surgery. However, in patients with Stage C colon cancer, the results of the intergroup trial[7] and the NCCTG trial[6] support the use of adjuvant therapy if it is initiated in the immediate postoperative period. Furthermore, it now appears unacceptable to include an untreated control group in future clinical studies in this population of patients. Whether such adjuvant treatment should consist of fluorouracil and levamisole, fluorouracil and folinic acid, fluorouracil and folinic acid and levamisole, or an alternative experimental regimen remains to be determined. Nonetheless, it is clear that the important trials described by Moertel et al. and the NCCTG researchers have added Stage C colon cancer to the list of malignant diseases in which adjuvant therapy can improve the chances of survival.

Dana–Farber Cancer Institute
Boston, MA 02115

ROBERT J. MAYER, M.D.

REFERENCES

1. Mayer RJ, O'Connell MJ, Tepper JE, Wolmark N. Status of adjuvant therapy for colorectal cancer. J Natl Cancer Inst 1989; 81:1359-64.
2. Buyse M, Zelemuch-Jacquotte A, Chalmers TC. Adjuvant therapy of colorectal cancer: why we still don't know. JAMA 1988; 259:3571-8
3. Janssen PA. The levamisole story. Prog Drug Res 1976; 20:347-83
4. Amery WK. Clinical experience with adjuvant levamisole in controlled cancer trials. In: Serrou B, ed. Current concepts in human immunology. New York: Elsevier, 1982:585-92.
5. Verhaegen H, De Cree J, De Cock W, Verhaegen-Declerq ML, Verbruggen F. Levamisole therapy in patients with colorectal cancer. In: Terry WD, Rosenberg SA, eds. Immunotherapy of human cancer. New York: Excerpta Medica, 1982:225-9.
6. Laurie JA, Moertel CG, Fleming TR, et al. Surgical adjuvant therapy of large bowel carcinoma: an evaluation of levamisole and the combination of levamisole and fluorouracil: the North Central Cancer Treatment Group and the Mayo Clinic. J Clin Oncol 1989; 7:1447-56.
7. Moertel CG, Fleming TR, Macdonald JS, et al. Levamisole and fluorouracil for surgical adjuvant therapy of colon carcinoma. N Engl J Med 1990; 322:352-8.
8. Arnaud JP, Buyse M, Nordlinger B, et al. Adjuvant therapy of poor prognosis colon cancer with levamisole: results of an EORTC double-blind randomized clinical trial. Br J Surg 1989; 76:284-9.
9. Buroker TR, Moertel CG, Fleming TR, et al. A controlled evaluation of recent approaches to biochemical modulation or enhancement of 5-fluorouracil therapy in colorectal carcinoma. J Clin Oncol 1985; 3:1624-31.
10. Green JL, Allegra CJ. Toxicity of levamisole and 5-fluorouracil in human colon carcinoma cells. J Natl Cancer Inst 1989; 81:1413-7.
11. Windle R, Bell PR, Shaw D. Five year results of a randomized trial of adjuvant 5-fluorouracil and levamisole in colorectal cancer. Br J Surg 1987; 74:569-72.

CORRESPONDENCE

THE CHERNOBYL NUCLEAR ACCIDENT

To the Editor: Cassel and Leaning began their editorial (July 27 issue)[1] with a review of the report on the Chernobyl nuclear accident by Baranov et al.[2] in the same issue, but they very quickly shifted to a barrage of warning shots across the bow of

EXHIBIT G

JAB 339

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

Inventors : Josephus Brugmans, William Pollack, Paul A. J. Janssen, and Daniel Tripodi

U.S. Patent No.: 4,584,305

Issued: April 22, 1986

For: AIDING THE REGRESSION OF NEOPLASTIC DISEASE WITH 2,3,5,6-TETRAHYDRO-6-PHENYLAMIDAZO[2,1-b]THIAZOLE

Hon. Commissioner of Patents and Trademarks
Box Patent EXT
Washington, D.C. 20231

CERTIFICATION

I hereby certify that this Application for Extension of Patent Term Under 35 U.S.C. 156 as well as all Exhibits A through H thereto is being submitted in duplicate to the Commissioner of Patents and Trademarks, Box Patent EXT, Washington, D.C. 20231

Date: July 31, 1990

Charles J. Metz

Charles J. Metz
Registration No. 20,359
Attorney for Applicants

Johnson & Johnson
One Johnson & Johnson Plaza
New Brunswick, NJ 08933-7003
(201) 524-2814
August 1, 1990

STATE OF NEW JERSEY)
) ss.
COUNTY OF MIDDLESEX)

BE IT REMEMBERED, that on this 31st day of July, 1990, before me, a Notary Public, personally appeared Charles J. Metz, who I am satisfied is the person named in and who executed the foregoing instrument in my presence, and I having first made known to him the contents thereof, he did acknowledge that he signed, sealed, and delivered the same and his voluntary act and deed for the uses and purposed therein expressed.

Ruth Ann Kreiger
Notary Public

RUTH ANN KREIGER
NOTARY PUBLIC OF NEW JERSEY
My Commission Expires November 11, 1994

EXHIBIT H

JAB 339

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

Inventors : Josephus Brugmans, William Pollack, Paul A. J. Janssen, and Daniel Tripodi

U.S. Patent No.: 4,584,305

Issued: April 22, 1986

For: AIDING THE REGRESSION OF NEOPLASTIC DISEASE WITH 2,3,5,6-TETRAHYDRO-6-PHENYLAMIDAZO[2,1-b]THIAZOLE

Hon. Commissioner of Patents and Trademarks
Box Patent EXT
Washington, D.C. 20231

DECLARATION

Dear Sir:

I, Charles J. METZ, residing at 15 Bellegrove Drive, Upper Montclair, New Jersey 07043 , declare as follows:

1) THAT I am a Patent Attorney authorized to practice before the United States Patent and Trademark Office (registration number 20,359) and have general authority to act in patent matters before the United States Patent and Trademark Office on behalf of Janssen Pharmaceutica N.V., the owner of the above-identified patent for which term extension is being requested.

2) THAT I have reviewed and understand the content of the application for patent term extension which is submitted pursuant to 35 U.S.C. 156 of which the present Declaration is attached as Exhibit H.

3) THAT I believe that U.S. Patent 4,584,305 is subject to extension pursuant to 37 C.F.R. 1.710.

4) THAT I believe an extension of one year, one month, and twenty-seven days of the term of U.S. Patent 4,584,305 is justified under 35 U.S.C. 156 and the applicable regulations.

5) THAT I believe U.S. Patent 4,584,305 for which the extension is being sought meets the conditions for extension of the term of a patent as set forth in 37 C.F.R. 1.720.

I hereby declare that all statements made herein of my own knowledge are believed true and tht all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so make are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application and any extension of U.S. Patent 4,584,305.

Charles J. Metz

Date: July 31, 1990 Charles J. Metz

STATE OF NEW JERSEY)
) ss.
COUNTY OF MIDDLESEX)

BE IT REMEMBERED, that on this 31st day of July, 1990, before me, a Notary Public, personally appeared Charles J. Metz, who I am satisfied is the person named in and who executed the foregoing instrument in my presence, and I having first made known to him the contents thereof, he did acknowledte that he signed, sealed, and delivered the same and his voluntary act and deed for the uses and purposed therein expressed.

Ruth Ann Kreiger

Notary Public
RUTH ANN KREIGER
NOTARY PUBLIC OF NEW JERSEY
My Commission Expires November 11, 1994

JAB 339

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

Inventors : Josephus Brugmans, William Pollack, Adi Paul A. J. Janssen, and Daniel Tri[illegible]

U.S. Patent No.: 4,584,305

Issued: April 22, 1986

For: AIDING THE REGRESSION OF NEOPLASTIC DISEASE WITH 2,3,5,6-TETRAHYDRO-6-PHENYLAMIDAZO[2,1-b]THIAZOLE

Hon. Commissioner of Patents and Trademarks
Box Patent EXT
Washington, D.C. 20231

TRANSMITTAL LETTER

Dear Sir:

Transmitted herewith is an Application for Extension of Patent Term under 35 U.S.C. 156 in the above-identified patent.

Please charge the $550.00 application fee to Deposit Account No. 10-750 in the name of Johnson & Johnson.

The Commissioner is hereby authorized to charge any additional fee which may be required or to credit any overpayments to Deposit Account 10-750.

Two copies of this letter are enclosed.

Respectfully submitted,

Charles J. Metz

Charles J. Metz
Registration No. 20,359
Attorney for Applicants

Johnson & Johnson
One Johnson & Johnson Plaza
New Brunswick, New Jersey 08933-7003
(201) 524-2814

July 31, 1990